Fluorometric Determination of Thallium with Rhodamine B

By Hiroshi ONISHI

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After a study of a photometric (absorptiometric) determination of traces of thallium with rhodamine B[1,2], it was thought worthwhile to attempt a fluorometric determination of the element by making use of the same rhodamine B-benzene extraction technique. The orange-yellow fluorescence of the benzene extract of rhodamine B chlorothallate was already used by Feigl, Gentil, and Goldstein[3] for the detection of thallium.

Experimental

Apparatus. — A Hitachi EPV-2 spectrophotometer with a fluorescence attachment, type L-2, was used. It was equipped with a Toshiba SHL-100 UV lamp and a filter to transmit the 365 mμ mercury line. One-centimeter glass cells of which four sides are transparent, were used.

Solutions. — *Rhodamine B*, 0.0020% (W/V). This was chosen as a reference standard to adjust the instrument to a given sensitivity. Other solutions used were the same as described before[2].

Procedure.—The separation of thallium and the rhodamine B-benzene extraction were made essentially according to the directions previously described[2]. In establishing the standard curve, however, evaporation of standard thallium solution and 0.50 ml. of 1 : 5 sulfuric acid to fumes followed by the treatment with 5.0 ml. of 2.0 N hydrochloric acid and 1.0 ml. of saturated bromine water is recommended.

Measurement of the fluorescence intensity. Set the wave length scale at 580 mμ. For determining 1 to 10 μg. of thallium, set the sensitivity of the instrument at 10 (the highest sensitivity), and adjust the scale reading with 0.0020% rhodamine B solution to 100%. Measure the fluorescence intensity of the sample solution immediately.

Results and Discussion

The fluorescence spectra of aqueous rhodamine B solution[4] and rhodamine B chlorothallate in benzene are given in Figures 1 and 2. The fluorescence peaks of rhodamine B and rhodamine B chlorothallate are found at about 575 and 580 mμ respectively. As the slit widths used were large, these figures are somewhat approximate. However, the two curves are so similar that rhodamine B solution has been adopted as a reference standard to adjust the fluorometer scale. If total fluorescence measurements were made, other reference standards such as quinine sulfate could be used.

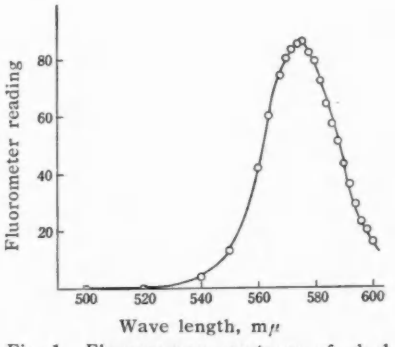

Fig. 1. Fluorescence spectrum of rhodamine B solution. 0.0020%, 1-cm. cell, slit width = 0.28 mm.

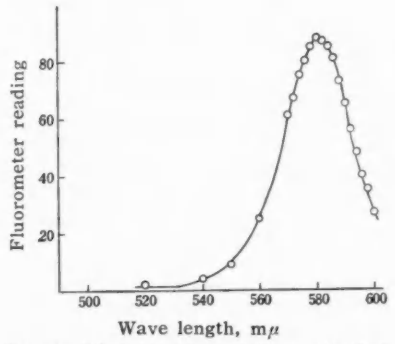

Fig. 2. Fluorescence spectrum of rhodamine B chlorothallate in benzene. 10 μg. Tl taken, 1-cm. cell, slit width = 0.36 mm.

In the present work, effects of variables such as the concentration of hydrochloric acid and rhodamine B were not particularly studied, and the conditions developed for the absorptiometry were accepted. A working curve is shown in Fig. 3. Curve

1) H. Onishi, This Bulletin, **29**, 945 (1956).
2) H. Onishi, This Bulletin, **30**, 567 (1957).
3) F. Feigl, V. Gentil, and D. Goldstein, *Anal. Chim. Acta*, **9**, 393 (1953).
4) For a more concentrated rhodamine B solution, see A. Murata and F. Yamauchi, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **77**, 1259 (1956).

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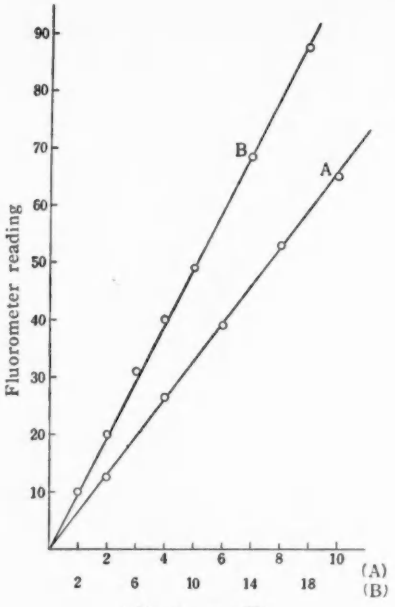

Fig. 3. Fluorescence intensity-concentration curve of thallium. Reference standard set at 100 for curve A, and at 70 for B, temperature 14—17°C.

A was obtained in setting the reference standard (0.0020% rhodamine B solution) at 100, whereas curve B was obtained at 70. It was observed that the fluorescence intensity of both rhodamine B solution and the benzene extract of rhodamine B chlorothallate decreased as the temperature was raised*. Consequently, working solutions as well as the sample should be at the same temperature. With the fluorometer used, the measurement of fluorescence intensity was made immediately after transferring the reference standard and the sample to the cell compartment. Fig. 3 shows that a satisfactory standard curve can be obtained by a careful and quick measurement. By decreasing the sensitivity and slit width of the instrument, larger amounts of thallium may be determinable. This point has not, however, been tested.

Effect of other elements on the photometric determination of thallium was previously described[2]. No serious interference was found by the elements studied. In fluorometry, it is not very uncommon that the presence of foreign substances sometimes interferes with the determination in a peculiar way, e. g., quenching. By carrying out the dithizone-chloroform extraction from basic solution containing cyanide, only lead, bismuth, and tin(II) are extracted with thallium (I). However, these elements in small quantities do not interfere with the fluorometric determination of thallium (Table I).

* With the instrument used (Hitachi EPV-2 with fluorescence attachment), the cell compartment was not well insulated from the heat of the light source.

TABLE I

DETERMINATION OF THALLIUM IN SYNTHETIC MAFIC ROCK*

Addition	Tl added, p.p.m.	Tl found, p.p.m.
none	none	0.15, 0.1
none	3.0	3.1, 2.6
0.1 mg. each Pb, Bi, Sn	none	0.1, 0.1
0.1 mg. each Pb, Bi, Sn	3.0	3.0, 2.8

* Percentage composition: 48 SiO_2, 12 Al_2O_3, 15 Fe_2O_3, 10 MgO, 10 CaO, 3 (Na_2O+K_2O), 1.5 TiO_2, 0.3 P_2O_5, 0.25 MnO.

A 1.0 g. sample simulating a mafic rock (10 per cent Fe) was decomposed with sulfuric- hydrofluoric acid, and the excess of sulfuric acid was driven off. The residue was taken up in water and the procedure for the dithizone extraction[2] was followed. Rhodamine B chlorothallate was extracted with 10 ml. of benzene after addition of 1 ml. of 0.20% (W/V) rhodamine B solution. In the table, +10% correction suggested previously, is not applied.

Although the present work has demonstrated the feasibility of a fluorometric determination of thallium, it does not appear that the method has any distinct advantages over the absorptiometric method. In sensitivity, the absorptiometric method is probably superior to the fluorometric. In accuracy, there may not be much difference between the two. The fluorometry is more susceptible to temperature.

Summary

A method is presented for the fluorometric determination of traces of thallium. Except for the step of fluorometry, the procedure is essentially the same as previously described for the absorptiometry. A synthetic mafic rock was analyzed for thallium by the method which involves hydrofluoric acid decomposition of the sample, dithizone extraction, rhodamine B-benzene extraction, and the measurement of fluorescence intensity on the benzene extract.

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The Leakage Method and the Sandwich Type Zisman Method for Measurements of Contact Potential Differences between two Metals Immersed in Poorly Conductive Dielectric Liquids

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(Received March 12, 1957)

The first measurement of contact potentials for metals immersed in a dielectric liquid was reported by H. J. Plumley[1], using a modified Kelvin method, in connection with the problem of the origin of the electrical conductivity of non-polar dielectric liquids. Since then no report has appeared on this subject except those by J. T. Davies[2] who measured the contact potentials at the oil-water interface by applying the Zisman method. Still there remain some important problems to be solved in their measurements. Quite independently of them our efforts have been devoted for several years to extend the Zisman's vibrating electrode method[3] to liquid phase; our intention has been to apply the measurement of contact potential difference (abbreviated to C.P.D.) or surface potential difference as a powerful tool to the research of surface chemical phenomena occurring at metal surfaces immersed in dielectric liquids. The reports on the technique of the measurement[4] and on the distinction between "true C. P. D." and "apparent C. P. D."[5-7] were published previously. The measured values of the C. P. D. between platinum and silver in various dielectric liquids were also reported[8].

Although various methods were reported for the measurement of C. P. D. in vacuo and in gaseous media, the Zisman method has been most widely used because of its convenience especially in gas phase. At an early stage of this research "immersion type Zisman method" was used for the purpose of measuring the C. P. D. between two metals immersed in a dielectric liquid. During the course of this study, it was found that the decrease in the C. P. D. was often caused by the passage of leakage current in the case of conductive dielectric liquids. Therefore, at the present time "sandwich type Zisman method" is recommended as the most suitable one in poorly conductive dielectric liquids. The immersion type Zisman method should be applied only in the dielectric liquids having a very high leakage resistance such as benzene.

The Zisman method, however, is accompanied by a disadvantage, which is that we have to compel one of the two electrodes to vibrate. Vibration of the electrode often brings about troublesome problems in the construction of the apparatus in vacuo or in dielectric liquids. In our laboratory, another new method without electrode vibration for measuring the C. P. D. in dielectric liquids having electrical conductivity was established by employing an electrometer and was named "leakage method". In order to measure C. P. D. without vibrating the electrode, generally, the medium between the two electrodes must have a certain degree of electrical conductivity. In this new method, a small conductivity which is inherent in the liquid was utilized without any additional ionization. Provided that the charge carriers of leakage current do not obey Faraday's laws of electrolysis, the C. P. D. in the liquid can be measured easily by this new method which resembles, in its operation, the ionization method. The ionization method in gas phase has been well known, and that in liquid phase for measuring the interfacial potentials of oil-water was already described by J. T. Davies[9]. But this leakage method utilizing the small electrical conductivity in herent in the liquid is not only entirely new but also interesting. By the analysis of the

1) H. J. Plumley, *Phys. Rev.*, **52**, 140 (1937).
2) J. T. Davies, *Nature*, **167**, 193 (1951); J. T. Davies, *Z. Elektrochem.*, **55**, 359 (1951).
3) W. A. Zisman, *Rev. Sci. Instr.*, **3**, 367 (1932).
4) A. Kanamori, *Bull. Nagoya Inst. Tech.*, **6**, 261 (1954).
5) Y. Yashiro and A. Kanamori, *J. Appl. Phys., Japan*, **23**, 129 (1954); Y. Yashiro, *Bull. Nagoya Inst. Tech.*, **6**, 281 (1954).
6) Y. Yashiro, ibid. **50th** Anniversary, 343 (1955).
7) A. Tanaka, ibid., **8**, 293 (1956).
8) Y. Yashiro, *J. Appl. Phys., Japan*, **24**, 144 (1955).
9) J. T. Davies, *Trans. Faraday Soc.*, **49**, 683 (1953)

experimental conditions of the two measurement methods, it became clear that, in order to compare directly the values of the C. P. D. obtained by this leakage method with those by the Zisman's, electrical resistance of the liquid between the two electrodes must be approximately 10^7—$10^{12}\,\Omega$. Some of the experimental evidences were also described.

Principles of the Measurement

For the first place, a case of the leakage method is illustrated. A liquid having suitable value of leakage resistance is sandwiched between the metal electrodes A and B as seen in Fig. 1. Under the condition that there exists a C. P. D. between the electrodes, leakage current i will flow through the liquid owing to the electrical field arising from the C. P. D. It is desirable that this current flow through the liquid is kept as small as possible throughout the measurement, because there is a possibility that so much of leakage current flow may cause the change in the C. P. D. In Fig. 1, as an excellent null indicator, a dynamic condenser electrometer is employed together with a cathode ray oscilloscope. The C_c is a coupling condenser whose insulation resistance is infinite. Assuming that each of the two electrodes is in a uniform surface state, the dynamic condenser of the electrometer C_e will be charged up by the C. P. D. until the voltage between the points E and F, V_{EF}, just balances the C. P. D. as shown in the round brackets. Then there is no potential difference between A and B, and no current flows*.

For the purpose of measuring this voltage, V_{EF}, this d. c. voltage is converted into an a. c. signal by means of the vibration of C_e, then this a. c. signal is amplified and finally connected to an indicator, e. g. a cathode ray oscilloscope. Under the condition that the voltage between the points E and F, V_{EF}, caused by the C. P. D. is just compensated through the liquid and the input resistor R_i with the aid of the potentiometer P. M. as shown in the square brackets, the input signal to the amplifier must become zero and so the oscilloscope exhibits no signal. Consequently we can determine the C. P. D. between the electrodes A and B from the difference of the potentiometer readings obtained by the change-over of the switch S_1 as in the usual case. The C. P. D. has the same magnitude as the difference of the potentiometer, but with the opposite sign.

At this stage of explanation, we call our attention to the fact that, if we suppose that these two electrodes A and B are the electrodes of an ordinary chemical cell, A is the cathode and B is the anode in the present instance (Fig. 1). On the other hand, when we consider that this voltage is exclusively caused by the C. P. D. between A and B, A is positive with respect to B. However, at the present stage of this research the distinction between electrochemical electromotive forces[10] and C. P. D. which is supposed by the author, is not yet well known in these fields and will become clearer with the progress of this research.

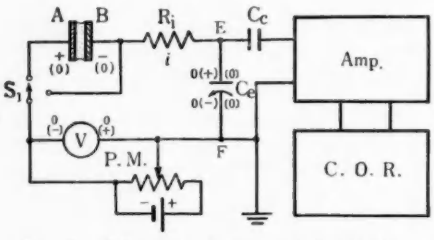

Fig. 1. Schematic diagram of the leakage method.
A, B=the metal electrodes. R_i=input resistor. i=leakage current. C_c=coupling condenser. C_e=dynamic condenser of the electrometer. S_1=switch. V=voltmeter. P. M.=potentiometer. Amp.=amplifier. C. O. R.=cathode ray oscilloscope.

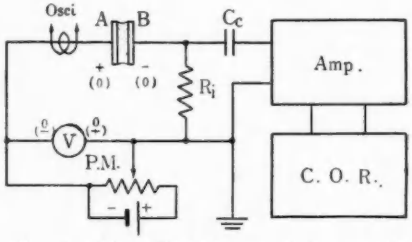

Fig. 2. Schematic diagram of the sandwich type Zisman method.

For the second place, in order to measure the C. P. D. by applying the Zisman method, we connect the circuit as shown in Fig. 2. When one of the two electrodes, A or B,

* The condition of surface uniformity will be important to measure accurately the C. P. D. or the surface potential difference by the ionization method. Otherwise, errors will probably be introduced.

10) A. Gemant, *Trans. Electrochem. Soc.*, **78**, 49 (1940); A. von Hippel, "Dielectric Materials and Applications", The Technology Press of M. I. T. and John Wiley & Sons, Inc., New York (1954), p. 165.

is vibrated by means of an audio frequency oscillator, an a.c. signal will be generated across input resistor R_i and amplified as in the ordinary case. Under the condition that the C. P. D. is just compensated by the potentiometer as shown in the parentheses, the input to the amplifier will vanish. The C. P. D. must be equal to the reading of the potentiometer, but with the opposite sign in this case again.

Moreover, when the surface state of each electrode is uniform, when the liquid is held between the electrodes A and B like a sandwich, and when the C. P. D. is cancelled by adjusting the voltage of the potentiometer, then the potential difference between the electrodes A and B will disappear and no leakage current flows even though the liquid has electrical conductivity. As shown in our experiments[5], in which the apparent C. P. D. is distinguished from the true C. P. D., the spurious increase in the C. P. D. will not arise in this case. Therefore, the measured values of the C. P. D. using this sandwich type Zisman method must be equal to the true C. P. D. This conclusion was also confirmed experimentally even in nitrobenzene with such a small leakage resistance as that of the order $10^7\Omega$*. But, in this sandwich type Zisman method too, it is advisable to check this fact occasionally following the method reported previously[5].

Electrical Equipment and Apparatus

In this experiment to check directly the data of C. P. D. measured by this leakage method compared with those obtained by the sandwich type Zisman method, the electrical circuit was connected as seen in Fig. 3. With slight improvements, a dynamic condenser pH-meter (electrometer) was employed as the parts of dynamic condenser C_e and amplifier. A cathode ray oscilloscope was employed as an indicator and the potentiometer was the same as reported previously[6,11]. The operation to measure C. P. D. was illustrated above. Careful attention should always be paid to the shielding of the input circuit to amplifier. All the measurement was carried out by manual null method and the sensitivity of this equipment was within ± 2 mV.

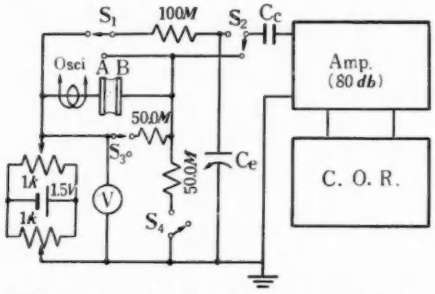

Fig. 3. Practical circuit used in the measurements.
S_1=switch, same as in Fig. 1. S_2, S_3=linked switch for the change-over of circuit from the sandwich type Zisman method to the leakage method, and vice versa. S_4=switch for measuring the true and the apparent C. P. D. Osci.=CR oscillator, operated at 150—160 c/s. C_e=500 pF polystyrene condenser.

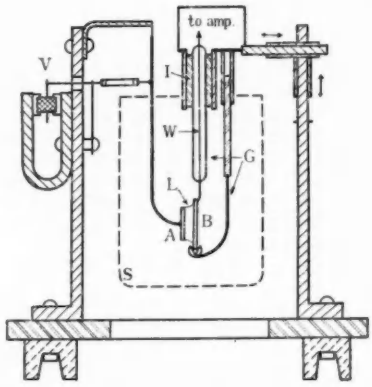

Fig. 4. Apparatus for mounting the electrodes.
V=vibrating reed of magnetic speaker. I=insulator. W=lead wire. G=glass supporter. A=Pt reference electrode. B=sample of metal. L=dielectric liquid. S=shielding.

The part of the electrodes was sketched in Fig. 4, which was designed for another experiment[4] and was used in this measurement too. A dielectric liquid was sandwiched between the two electrodes with a syringe. The distance between the electrodes was so small (0.5—1.0 mm) that the liquid neither dropped down nor evaporated within a short time except when the liquid had very high vapor pressure at room temperature.

Examples of the Measurement

Although electrical potential differences at metal-liquid interfaces will result from various origins, yet the electrical potential difference between two metal electrodes immersed in the dielectric liquids which satisfy the following conditions, should be called "contact potential

* Details of this experiment will be published in near future.

11) Y. Yashiro, *Kôbunshi*, **5**, 392 (1956).

difference" of the metals in the liquid according to the author's view. These requirements are as follows;—

1) The decomposition voltage of the liquid, if it exists, must be much higher than the C.P.D. between the two metals immersed in the liquid.

2) In case of the presence of electrical conductivity, the charge carriers of leakage current do not cause chemical reactions with the electrodes by its passage through the liquid. Nevertheless, there may be a case that these carriers cause the change in the C. P. D. by the space-charge polarization arising from the selective accumulation of the carriers at metal-liquid interfaces, when the current is flowing through the liquid under the influence of external electrical field; but this is not involved in the present requirement. Therefore, the term "chemical reaction" should be accepted in its narrower sense and the C. P. D., in its broader sense[1] in this statement.

In addition, in order to compare the data obtained by the leakage method with those by the sandwich type Zisman method, it is desirable to choose a liquid having a leakage resistance ranging 10^7—$10^{12}\Omega$ as a sample of measurement for the reasons discussed below. In the present measurement, nitrobenzene and *o*-dichlorbenzene were used.

TABLE I

COMPARISONS OF THE DATA OBTAINED BY THE TWO METHODS

Dielectric liquid	Sample of metal	The leakage method	The sandwich type Zisman method
Nitrobenzene	Al	+500(mV)	+505(mV)
	Cu	+220	+220
	Ni	+330	+340
o-Dichlorbenzene	Al	+470	+470
	Cu	+120	+130
	Ni	+415	+410

Nitrobenzene and *o*-dichlorobenzene of chemical pure grade were used after two redistillations. The leakage resistance of the former was $10^7\Omega$ and that of the latter was $10^8\Omega$ under the condition of this measurement. All the metals used were of commercial quality and in the form of a plate (2 cm×2 cm×0.5 mm). These plates were polished in the air with clean gauze and purified carborundum powder. The polished-up platinum plate was used as a reference electrode. Within some time from the beginning of the measurement, the C. P. D. values settled into a constant value, then both values of the C. P. D. were obtained by the two methods respectively following the operations described above.

In this measurement, individual values of the C. P. D. were not of great concern, but the degree of agreement of the results by the two methods, was important. As seen in Table I, both data agreed well within the error ±10 mV. Usually the error of this extent will be inevitable at the present stage of this experiment. These samples were all positive with respect to the platinum reference and the data were obtained at room temperature.

Discussion on the Limit of Measurement

The limit that the C. P. D. can be measured by the leakage method is determined mainly by the magnitude of the time constant of the input circuit. Fig. 5 shows

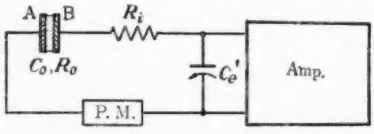

Fig. 5. Equivalent circuit of the leakage method.
C_o=capacitance between A and B. R_o =leakage resistance of the liquid. R_i input resistor, usually 10^8—$10^{10}\Omega$. C_e'= average capacitance of the dynamic condenser plus stray capacitances. Amp.= ideal amplifier, i. e., one whose input impedance is infinite. P. M.=potentiometer, whose internal resistance $R_{P.M.} \ll R_o$ or R_i.

an equivalent circuit of the leakage method, and this belongs to a *CR* circuit. Representing the sum of R_o and R_i by R_s, and that of C_o and C_e' by C_s, the time constant of this *CR* circuit should be $\tau_s = C_s \times R_s$. If this time constant becomes too large, it is practically impossible to measure C. P. D. because too long a time is taken for charging up the dynamic condenser C_e. Considering that the minimum value of C_s experimentally chosen will be, at the best, roughly ten *pF* and that the maximum value of τ_s permitted by practical operations will be several seconds, R_s or R_o should be smaller than the order of $10^{12}\Omega$ where $R_o \gg R_i$. In this method, however, no limitation is required on the lower range of R_o.

In the sandwich type Zisman method (Fig. 2), however, the lower limit does exist but the upper limit does not. As the leakage resistance of the liquid becomes smaller, the a. c. voltage generated by the electrode vibration decreases correspondingly and, at last, becomes comparable to the intrinsic noise of the amplifier; in other words, the lower limit of R_o will be determined mainly by the amplifier sensitivity. Full analysis of this problem is so complicated that it is beyond our scope in this experiment. Perhaps it will be sufficient for the present purpose

to determine this lower limit experimentally on the simplest condition. When all the electric circuit constants remain unchanged except R_0, the lower limit of R_0 is roughly $10^7\Omega$ in the equipment shown in Fig. 3.

Therefore, only in the liquid having leakage resistance of 10^7—$10^{12}\Omega$ or specific resistance of 10^8—$10^{13}\Omega\cdot$cm, can the measured values of the C. P. D. by the leakage method and by the sandwich type Zisman method, be compared with each other.

Summary and Conclusion

For the purpose of measuring the C. P. D. between two metals immersed in poorly conductive dielectric liquids, two methods were presented in this report. One is the leakage method, which appears to resemble the ionization method but is different in one point; because in this leakage method we make use of the small electrical conductivity inherent in the liquid. It is another merit of this method that the measurement can be carried out without vibrating the electrode. When the liquid and the electrodes are properly chosen, this leakage method may be applied to various purposes. The other is the sandwich type Zisman method, which is an excellent device of improvement of the vibrating electrode method in dielectric liquids. On the comparison of the measured values by the two methods, satisfactory agreements were obtained.

Of course, there is only limited knowledge at the present time on the nature of the electrical potentials observed in these dielectric liquids, yet the author ventures to say that the use of the term, "contact potential difference", is most suitable in the case of these experiments.

The author wishes to express his hearty thanks to Assist. Professor Y. Yashiro of this Institute for his many valuable suggestions and his constant interest throughout the present work.

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Studies on Inorganic Constituents in Biological Materials.—On the Inorganic Constituents in Human Stones

By Naoichi OHTA

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Human stones, or calculi, are mainly formed in the liver, gall-bladder, biliary duct, pancreas, kidney, urethra, bladder, etc. in consequence of the isolation and coagulation of their respective humours caused by various reasons. Although many studies on calculi have been undertaken hitherto, most of them are dealing with etiological or clinical problems, and with all those studies no perfect explanation in the field has yet been made. Concerning the chemical composition of calculi, a long time has passed since their major constituents were made known, but there are only a few studies that deal with the inorganic constituents of calculi systematically. Recently, owing to developments in the instrumental method of analysis, many elements have been detected in calculi, and discussions on the significance of those elements have become active. However, all the analyses whcih have ever been made are limited almost entirely to qualitative research, or even if quantitative, to only a few constituents.

Regarding the formation of calculi as a special chemical phenomenon, the author considers that on the one hand it is important to determine etiologically the contents of inorganic constituents in calculi which are formed in various organs and their respective humours of a human body and to reveal the status of distribution of those constituents in those materials, and that on the other hand it is a matter of interest to clarify the cycle and function of elements in biological materials. The author[1] has reported his studies on each of the constituents of human stones several times up to this time. In this paper, he will report (1) the results of

1) N. Ohta, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **74**, 506 (1953); **76**, 590, 1235 (1955); **78**, 7, 13, 1033, 1038, 1043 (1957).

quantitative analyses of various human stones and of the humours in which the stones were formed, determining calcium, magnesium, phosphorus, sodium and potassium as major inorganic constituents and iron, copper, zinc, lead and manganese as heavy metal constituents, (2) the distribution of those constituents in various human stones and in their respective humours, and, (3) the mutual relation existing among those constituents.

Experimental

Materials.—The human stones used for this study were 25 gallstones (since three of them were divided into the internal and the external portions, the total number of analyses was 28), 10 urinary calculi and two pancreatic calculi, all of them being (kindly) providedly by the First National Hospital of Tokyo. The characters of these materials have been described in the author's earlier reports[1]. The samples of related humours provided for the analyses were 10 of gall-bladder biles which had been collected at the time of the autopsies at Tokyo Medical Examiner Office, 12 of normal human blood and 12 of normal human urine.

Methods.—Samples were analysed after conversion of their organic matters into ashes with redistilled fuming nitric acid and 60 % perchloric acid.

Calcium and magnesium were determined by the complexometric titration method[2]. The sum of calcium and magnesium was titrated with disodium salt ethylendiamintetra-acetic acid solution using Eriochrom Black T as an indicator, while the calcium was determined indirectly by determining magnesium after precipitation of calcium as oxalate. On the analysis of samples rich in phosphate like urinary calculi, the cation exchanger, Dowex 50, was used for preliminary separation of the phosphoric acid radical. This method was also applied to the determination of lead and zinc in the samples rich in phosphate.

Sodium and potassium were determined by the flamephotometric method[3], using Perkin-Elmer Model-52C flamephotometer, acetylene being used as fuel.

Phosphorus was determined by molybdenum blue method modified by Ikeda[4].

Iron was determined by nitroso-R salt method modified by Dean and Lady[5].

Copper, zinc and lead were determined according to Sandell's dithizone method[6].

Manganese was determined by permanganate method[7].

Of the elements described above, last five were determined by Beckmann Model D. U. Quartz Spectrophotometer.

Results

Analytical results for gallstone, normal gallbladder bile, normal human blood, urinary calculus, normal human urine and pancreatic calculus were shown in Table I, II, III, IV, V and VI, respectively. In these tables, the values of the major and the heavy metal constituents were shown in the mg. per g. (ml.) and p. p. m. unit, respectively, of undried samples. "m" is an average value calculated statistically at the 95 % confidence interval.

Discussion

1. Major Constituents.—(*1*) *Gallstone.*—The chemical composition of a gallstone varies in accordance with the difference among human races and regions. The greater part of a gallstone consists of organic materials[8,9], such as cholesterin, bilirubin and fatty acid, and the content of inorganic constituents is very small, as shown in the ash content in Table I.

Although various gallstones have been classified clinically according to the content of above-mentioned organic constituents in them, most stones are either a mixture or a layer of several kinds (this shows the change of the circumstance in which the sedimentations were made), so that it is difficult to analyse a slight quantity of inorganic constituents after separating these components individually. Accordingly, the materials used in this study were divided into three groups, such as mineral stone, cholesterinpigment-calcium stone and cholesterin stone, and each sample of the gallstone was analysed as it was.

A cholesterin stone consists mainly of cholesterin (soluble in ether), and contains a slight amount of ash and bilirubin. A cholesterinpigment-calcium stone contains a considerable amount of bilirubin (soluble in chloroform) and calcium besides cholesterin. A mineral stone is scarcely soluble in chloroform and ether. It is either hard and contains a considerable amount of ash, or it has a black vitreous appearance.

The content of major constituents in various gallstones varies considerably according to these three kinds of stone

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3) C. E. Bills, F. G. McDonald, W. Niedermier and M. C. Schwartz, *Anal. Chem.*, **21**, 1076 (1949).
4) N. Ikeda, *J. Chem. Soc. Japan, Pure Chem. sec.*, **72**, 23 (1951).
5) J. A. Dean and J. H. Lady, *Anal. Chem.*, **25**, 947 (1953).
6) E. B. Snandell, "Colorimetric Determination of Traces of Metals", Interscience Publisher, Inc., New York, (1950), pp. 295, 616, 388.
7) E. B. Sandell, ibid., p.433.
8) M. Nishimura, *J. Biochem.*, **28**, 265 (1938).
9) K. Kodama, "Clinical Biochemistry", Nanzando, Tokyo, (1951), p. 117.

TABLE I

INORGANIC CONSTITUENTS IN HUMAN GALLSTONES

Species	Sample	Ash, %	Ca mg./g.	Mg mg./g.	Na mg./g.	K mg./g.	P mg./g.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
Mineral	G-4004	6.83	28.70	3.05	1.02	0.26	6.52	860	1,794	43	134	53
stone	G-4011	7.48	36.00	3.11	0.86	0.20	5.00	1,170	1,435	146	81	157
	G-4011B	12.57	31.82	4.37	—	—	21.30	2,290	10,400	385	84	277
	G-4012	9.12	29.24	4.52	1.10	0.19	10.60	598	8,705	1,043	97	140
	G-4017	15.56	70.40	21.00	0.77	0.17	4.20	250	1,665	108	47	98
	G-5007	8.45	39.20	9.41	0.93	0.21	4.01	—	2,107	168	33	—
	G-5012	8.75	34.00	10.70	1.34	0.21	5.65	411	1,355	423	45	66
	G-5017	32.96	132.	16.10	1.69	0.51	48.50	629	420	550	173	654
	G-5019	10.01	46.44	4.95	2.30	0.49	7.25	—	3,585	238	—	124
	G-5019A	8.26	35.31	3.71	—	—	9.32	68	1,382	279	43	83
	G-5019B	9.08	45.84	3.90	—	—	5.28	373	2,440	574	95	118
	G-5021	54.79	285.	35.52	0.31	0.09	28.10	287	548	159	51	540
	G-5022	36.89	173.	18.15	4.37	0.76	23.30	1,520	7,345	329	296	3,850
	G-8002	5.73	21.87	4.32	—	—	4.82	282	2,660	216	192	107
	Average	16.18	72.06	10.20	1.47	0.31	13.13	728	3,274	333	105	482
Chole-	G-1003	10.24	53.94	3.62	1.21	0.18	7.86	102	598	172	21	32
sterin-	G-1005	5.37	28.54	2.60	0.10	0.04	3.37	30	70	40	18	6
pigment-	G-4011A	6.95	34.92	3.27	—	—	4.77	61	281	116	46	33
calcium	G-5009	8.59	43.58	4.65	1.55	0.27	4.92	85	253	243	55	248
stone	G-5010	1.84	9.06	0.89	0.18	0.04	1.42	93	87	9	55	48
	G-5013	9.30	48.34	4.15	0.91	0.18	6.86	163	1,052	140	83	153
	G-5014	7.32	39.20	3.38	0.65	0.15	4.42	278	185	50	168	186
	G-5018	2.97	12.25	1.18	0.40	0.16	2.45	79	229	50	29	49
	G-8004A	1.33	5.37	0.42	—	—	1.82	71	255	149	51	55
	G-8004B	2.95	14.90	0.87	—	—	1.78	277	858	451	148	94
	Average	5.68	29.01	2.50	0.71	0.15	3.97	124	387	142	67	90
Chole-	G-4015	0.06	(0.03)	—	0.07	0.02	0.12	15	65	17	24	11
sterin	G-4016	0.04	(0.02)	—	0.07	0.02	0.04	31	35	58	28	6
stone	G-5001	0.08	(0.03)	—	0.15	0.08	0.14	13	35	67	24	11
	G-5015	0.04	(0.02)	—	0.06	0.02	0.06	29	17	79	—	10
	G-8001	0.03	—	—	—	—	—	16	27	12	23	10
	G-8003	0.44	0.94	0.13	—	—	0.94	133	80	105	79	27
	Average	0.12	0.21	0.13	0.09	0.04	0.26	40	43	56	36	13
m.		6.25	31.18	5.52	0.78	0.17	6.30	293	871	185	71	121
		±1.74	±12.04	±2.36	±0.30	±0.06	±2.71	±147	±383	± 61	±20	± 59

TABLE II

INORGANIC CONSTITUENTS IN POST-MORTEM GALLBLADDER BILE

Samples	Collected volume, ml.	Ca mg./ml.	Mg mg./ml.	Na mg./ml.	K mg./ml.	P mg./ml.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
No. 1	10.0	1.55	0.24	4.3	1.4	2.52	20.3	26.8	15.0	0.15	1.11
2	22.0	0.36	0.06	3.2	1.0	0.50	9.1	3.2	5.6	0.30	0.53
3	5.0	0.68	0.12	6.5	2.4	1.03	97.4	3.5	38.2	0.60	3.10
4	7.5	0.70	0.12	4.9	1.7	1.19	25.8	9.2	10.0	0.40	4.20
5	7.0	0.98	0.15	6.4	2.0	1.66	45.0	20.5	4.3	0.32	1.46
6	14.5	0.27	0.04	4.3	1.4	0.42	33.3	7.3	10.2	0.15	0.96
7	16.5	0.30	0.05	4.1	1.3	0.58	10.8	2.6	4.6	0.10	0.83
8	8.0	1.02	0.18	6.6	1.5	1.56	22.0	12.0	9.4	0.62	0.27
9	7.0	1.05	0.14	6.3	1.6	1.33	34.4	12.3	13.3	0.36	0.90
10	12.0	0.44	0.06	4.5	1.7	0.67	30.1	11.2	12.5	0.51	0.18
m.	11.0	0.74	0.12	5.1	1.6	1.15	25.6	10.9	9.4	0.35	1.35
		±0.30	±0.05	±0.9	±0.3	±0.47	± 8.8	± 5.5	± 3.0	±0.13	±0.93

TABLE III

INORGANIC CONSTITUENTS IN NORMAL HUMAN BLOOD

Sample	Ca mg./ml.	Mg mg./ml.	Na mg./ml.	K mg./ml.	P mg./ml.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
No. 1	0.127	0.053	2.18	2.26	0.20	530	1.31	10.9	0.28	0.45
2	0.133	0.055	2.50	2.20	0.20	482	1.26	8.44	0.25	0.36
3	0.114	0.038	2.09	1.62	0.16	480	1.15	7.79	0.29	0.37
4	0.083	0.032	1.81	1.43	0.10	390	0.89	5.50	0.22	0.28
5	0.105	0.043	2.36	1.96	0.18	488	1.10	6.50	0.30	0.25
6	0.130	0.053	2.40	2.08	0.23	542	1.28	7.82	0.36	0.46
7	0.111	0.045	2.47	1.81	0.18	474	1.13	8.60	0.27	0.27
8	0.103	0.045	2.33	1.90	0.16	469	1.17	7.97	0.25	0.30
9	0.087	0.037	2.00	1.99	0.16	498	1.24	8.06	0.34	0.32
10	0.094	0.040	2.34	2.05	0.22	522	1.22	8.25	0.33	0.48
11	0.090	0.037	2.34	2.01	0.16	477	1.19	7.21	0.30	0.45
12	0.082	0.036	2.15	2.09	0.15	470	1.15	7.11	0.32	0.39
m.	0.105	0.043	2.25	1.95	0.18	494	1.20	7.57	0.29	0.37
	±0.012	±0.005	±0.13	±0.15	±0.02	± 17	±0.04	±0.62	±0.03	±0.05

TABLE IV

INORGANIC CONSTITUENTS IN HUMAN URINARY CALCULI

Species	Sample	Ash, %	Ca mg./g.	Mg mg./g.	Na mg./g.	K mg./g.	P mg./g.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
Bladder	U-1001	17.09	52	7.0	4.30	8.14	30.5	270	4.8	345	36	1.0
calculus	U-5009	24.12	137	7.2	2.55	5.41	9.9	144	26.8	239	20	4.5
Renal	U-5002	58.16	286	12.7	4.57	0.52	63.3	256	17.8	913	43	4.1
calculus	U-5005	57.02	271	12.0	4.76	2.00	69.2	198	7.8	577	26	1.3
	U-5006	63.20	264	9.7	3.66	1.90	94.3	340	14.7	578	37	4.1
	U-5007	38.58	150	8.3	7.65	4.90	61.3	118	26.5	673	31	3.0
Urethral	U-5001	78.28	450	18.2	7.19	0.92	45.8	199	6.3	705	58	0.7
calculus	U-5003	51.67	233	3.8	4.59	1.41	70.5	286	8.7	343	19	1.0
	U-5004	37.37	254	1.5	1.69	0.98	3.3	172	16.9	561	22	1.7
	U-5008	62.61	184	31.4	4.70	2.71	120.	166	6.7	615	43	1.8
m.		48.82	228	11.2	4.57	2.89	56.8	215	13.7	555	34	2.3
		±13.66	± 77	± 6.1	±1.30	±1.77	±25.8	± 50	± 6.3	±142	± 9	±1.0

TABLE V

INORGANIC CONSTITUENTS IN NORMAL HUMAN URINE

Sample No.	Vol. ml./day	Average concentration mg./ml. Ca	Mg	Na	K	P	Sample No.	Vol. ml./day	Average concentration p.p.m. Fe	Cu	Zn	Pb	Mn
1	1480	0.116	0.052	3.84	1.14	0.64	7	1050	1.83	0.15	1.40	0.045	<0.01
2	2010	0.098	0.051	4.79	1.10	0.70	8	900	2.08	0.25	1.54	0.120	<0.01
3	1600	0.099	0.034	4.19	0.92	0.53	9	1000	2.40	0.22	1.45	0.080	<0.01
4	2000	0.077	0.035	3.20	0.76	0.55	10	1750	1.07	0.08	1.22	0.020	<0.01
5	1550	0.152	0.057	4.93	1.00	0.67	11	1200	1.66	0.14	1.82	0.052	<0.01
6	1630	0.127	0.049	1.74	0.51	0.73	12	1000	2.25	0.23	1.69	0.088	<0.01
Average		0.112	0.046	3.78	0.91	0.64	Average		1.88	0.18	1.52	0.068	<0.01

TABLE VI

INORGANIC CONSTITUENTS IN HUMAN PANCREATIC CALCULI

Sample	Ash, %	Ca mg./g.	Mg mg./g.	Na mg./g.	K mg./g.	P mg./g.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
P-1001	55.06	382	1.8	0.22	0.09	0.74	75.0	11.	282	136	0.3
P-4002	64.45	440	3.7	1.49	0.68	2.30	52.4	19.	345	112	0.5
Average	59.76	411	2.8	0.86	0.39	1.52	63.7	15.	314	124	0.4

as shown in Table I. The ratios of average content of ash between mineral, cholesterinpigment-calcium and cholesterin stones are 135 : 47 : 1, while the mutual ratios between each of the major constituents do not vary so much as those in ash content. As for the value (weight proportion) of Ca/Mg, that of a cholesterinpigment-calcium stone (12.0) is greater than that of a mineral stone (7.7) or of a cholesterin stone (7.2). As for the value of (Ca+Mg)/P, there is no significant difference between that of a mineral stone (7.5) and that of a cholesterinpigment-calcium stone (7.6), but the value of a cholesterin stone (0.5) is far smaller than the values of the other two. If calcium and magnesium that present (in the stones) as phosphates, the values of Ca/P and Mg/P would be 1.94 and 1.18, respectively. Accordingly, the value of (Ca+Mg)/P may be regarded as a standard for measuring the state of alkaline-earth metals. Epprecht et al.[10] reported in their experiment using X-ray that calcium in a gallstone was present as a bilirubinate, while Cameron et al.[11] reported the presence of calcium carbonate in a gallstone. As for the value of Na/K, there is no significant difference between that of a mineral stone (4.7) and that of a cholesterinpigment-calcium stone (4.5), while that of a cholesterin stone (3.0) shows that the proportion of potassium is greater than in the other two.

According to the result of an examination of relations existing among major constituents of a gallstone (as a whole), there are positive correlations between calcium and magnesium, sodium and potassium, phosphorus and sodium, and phosphorus and potassium, respectively, with 5% degree of significance.

(2) *Bile and Blood.*—Bile is a complexed colloidal solution containing bilirubine which is a decomposition product of hemoglobin. There are two kinds of bile, hepatic and gallbladder bile, the latter being concentrated 7 to 10 times as densely as the former[12]. A gallstone is nothing but the product of condensed bile constituents. Therefore, it is not only interesting to research the relation between inorganic constituents of bile and blood and those of gallstones in order to understand the cycle of elements in biological materials, but also it is important to clarify the mechanism of the formation of a gallstone.

The relationship between the formation of a gallstone and inorganic constituents mainly in regard to alkali and alkaline-earth metals has been studied previously, and there are several reports on the metabolism of those materials in humours obtained from experiments on animals[13-17].

When the content of potassium is taken as a standard, the ratio of existence of each of the constituents in blood, bile and gallstones are given in Table VII.

TABLE VII

	Ca	Mg	P	Na	K
Blood	0.054	0.022	0.092	1.2	1.0
Bile.....	0.46	0.075	0.72	3.2	1.0
Gallstone ...	183.	31.	37.	4.6	1.0

That is to say, gallstones are rich in alkaline-earth metals, especially in calcium and phosphorus, while blood and bile are rich in alkali metals.

When the content of each element in blood is taken as a standard, the ratios of the average content of each of the constituents in blood, bile and a gallstone are given in Table VIII (the figures shown in brackets indicate that the content of each of those constituents in bile is regarded as a standard).

TABLE VIII

Blood	Bile	Gallstone
Ca .....1.	7.0 (1.)	297. (42.)
Mg.....1.	2.8 (1.)	128. (46.)
P .....1.	6.4 (1.)	35. (5.5)
Na.....1.	2.3 (1.)	0.35 (0.15)
K .....1.	0.82(1.)	0.087(0.11)

We see then that calcium, magnesium and phosphorus increase according to the process of blood—bile—gallstone, while potassium decreases in the same process. Sodium increases in the process of turning from blood to bile, but the content of the element in a gallstone is less than that in blood. When the differences among solubilities of various salts composed of these metals, PO_4^{3-} and organic matters in a gallstone, and the relation between

10) W. Epprecht, H. Rosenmund and H. R. Schinz, *Fortshr. Gebiete Röntogenstrahlen*, **79**, 1 (1953); *C. A.*, **47**, 11458 (1953).
11) A. T. Cameron, F. D. White and S. Meltzer, *Can. Med. Assoc. J.*, **39**, 441 (1938); *C. A.*, **33**, 1811 (1939).
12) K. Kodama, "Clinical Biochemistry", Nanzando, Tokyo, (1951), p. 114.
13) R. Ohta, *J. Gastroenterology*, **2**, 1201 (1927).
14) T. Maruno, ibid., **6**, 642 (1931).
15) Y. Tachikawa, ibid., **10**, 1349, 1361, 1375 (1935).
16) T. Hukuda and Y. Tachikawa, ibid., **11**, 763 (1936).
17) R. Kono, *Jap. Arch. Internal Med.*, **1**, No. 2, 44, 49 (1954).

these metal ions and the stability[18] of the colloidal state of bile are considered, it is obvious that the abnormality of the metabolism of these major constituents has a strict connection with the formation of a gallstone.

All the values of Ca/Mg, (Ca+Mg)/P and Na/K increase according to the order of blood (2.4±0.1, 0.88±0.10 and 1.2±0.1), bile (6.4±0.5, 0.75±0.06 and 3.4±0.4) and gallstone (9.0±1.3, 5.8±1.3 and 4.3±0.6), because both the rate of increase of calcium and the rate of decrease of potassium are high.

(*3*) *Urinary Calculus and Urine.*—The history of research of a urinary calculus is long. Much literature on this subjects has been published since the end of the eighteenth century. According to the results of all the crystallographic studies using X-rays[19-23] and polarized microscope[24-29], chemical analysis[30-32] and infrared spectroscopic studies[33] ever made, it has been clarified that the urinary calculus contains one or more of the following compounds:

$Ca_3(PO_4)_2$, $CaHPO_4 \cdot 2H_2O$, $Ca_{10}(PO_4)_6(OH)_2$, $Ca_{10}(PO_4CO_3OH)_6(OH)_2$, $CaC_2O_4 \cdot H_2O$, $CaC_2O_4 \cdot 2H_2O$, $Mg_3(PO_4)_2$, $MgNH_4PO_4 \cdot 6H_2O$, carbonate, uric acid, NH_4 or Na acid urate, cystine, and xanthine.

Table IV gives the calculi assorted according to the parts where they had been formed, but the samples were so few that it was difficult to compare one with another. According to the result of analysis, it was ascertained that all of these calculi consisted of a large quantity of phosphate and oxalate of calcium and of a small quantity of magnesium compounds. According to Epprecht et al.[22], the genesis of $MgNH_4PO_4 \cdot 6H_2O$ in Europeans is more frequent than in Orientals.

As a whole, the ash content of urinary calculi is greater than that of gallstones, but there is no significant difference between them, so far as the mutual ratio of major constituents is concerned, except that the magnesium content is less. Therefore, regarding the values of (Ca+Mg)/P and Na/K, there is no significant difference between urinary calculi (5.1±3.4 and 3.0±2.1) and gallstones with the exception that Ca/Mg in urinary calculi (23.2±12.3) is greater than that in gallstones.

Urine is usually in a persaturate state against its solute, which may be crystallized to a urinary calculus owing to various reasons. Therefore, the relation between the formation of a urinary calculus and the colloidal character of urine has been observed for a long time[34-38].

The concentrations of calcium, magnesium, sodium, potassium and phosphorus in urinary calculus are 2036, 265, 1.2, 3.2 and 89 times, respectively, as much as those in urine. When the content of potassium is taken as a standard, the ratio of existence of each of the constituents in urinary calculus and urine are given in Table IX.

TABLE IX

	Ca	Mg	Na	K	P
Urinary calculus...	78.8	3.9	1.6	1.0	19.7
Urine	0.12	0.05	4.2	1.0	0.7

Thus urinary calculi are rich in alkaline-earth metals and phosphorus, while urine is rich in alkali metals.

The Values of Ca/Mg and (Ca+Mg)/P increase according to the process of urine (2.4 and 0.25)—urinary calculus (there are, however, a great differences between urinary calculi and gallstones in regard to the enrichment of calcium and magnesium), but there is no significant difference between them in regard to the value of Na/K (urine is 4.1).

(*4*) *Pancreatic Calculus.*—Both of the two examples have a greater amount of

18) T. Yuta, *Acta Medica* (IGAKU KENKYU), **23**, 1434 (1953).
19) J. A. Barclay, W. T. Cooke, M. Stacey, A. D. Booth and P. W. Kent, *J. Physiol.*, **103**, 249 (1944); *C. A.*, **39**, 978 (1945).
20) E. Brandenberger, H. R. Schinz and Fr. de Quervain, *Experimentta*, 3, 106 (1947); *C. A.*, **41**, 4560 (1947).
21) S. Fukushima, *Fukuoka Acta Medica*, **40**, 95 (1949).
22) W. Epprecht and H. R. Schinz, *Schweiz. med. Woch.*, **80**, 792 (1950); *C. A.*, **45**, 751 (1951).
23) J. Barraud, *Bull. Soc. Franç. Mineral.*, **75**, 166 (1952); *C. A.*, **46**, 6725 (1952).
24) A. Randell, IV., *J. Urol.*, **48**, 642 (1942); *C. A.*, **37**, 1455 (1943).
25) E. L. Prien and C. Frondel, *J. Urol.*, **57**, 949 (1947).
26) E. L. Prien, ibid., **61**, 821 (1949).
27) T. Sekimura, *Jap. J. Urol.*, **40**, 113, 116 (1949).
28) M. Ishibashi, *J. Kurume Med. Assoc.*, **13**, 48 (1950).
29) J. Boissier, *Ann. Biol. Clin.*, **10**, 523, 572 (1952); *C. A.*, **47**, 5474, 5529 (1953).
30) L. W. La Towsky, *J. Urol.*, **49**, 720 (1943); *C. A.*, **37**, 5094 (1943).
31) T. Sekimura, *Jap. J. Urol.*, **36**, 319 (1944).
32) E. D. Carpenter, *Can. J. Med. Technol.*, **16**, 53 (1954); *C. A.*, 10894 (1954).
33) D. E. Beischer, *J. Urol.*, **73**, 653 (1955).
34) I. Yoshikawa, *Acta Medica* (IGAKU KENKYU), **19**, 269 (1949).
35) L. Aschoff, *Kolloid Z.*, **89**, 107 (1939).
36) I. Palócz, *Orvosok Lapja Népegészségügy*, **2**, 1473 (1946); *C.A.*, **42**, 8926 (1948).
37) K. Kodama, "Clinical Biochemistry", Nanzando, Tokyo, (1951), p. 350.
38) E. A. Hauser and D. S. Le Beau, *Kolloid Z.*, **132**, 78 (1953).

ashes than in other sorts of human stone, and they are almost entirely composed of calcium carbonate. They contain only a small quantity of phosphates. Butturini and Meduri[39] reported similar results.

For this reason, the ratios of existence among those major constituents are (as K=1); Ca : Mg : Na : K : P=1054 : 7.2 : 2.2 : 1 : 3.9 and the mutual ratios are : Ca/Mg=166. (Ca+Mg)/P=35.6.

These values differ considerably from those of urinary calculi and gallstones.

2. Heavy Metal Constituents.—(*1*) *Gallstone, Bile and Blood.*—Heavy metal constituents, such as copper, iron, zinc and manganese are contained in gallstones, especially in mineral stones, much greater in quantity than in any other biological materials. Therefore, in several examples the amount of these metals even exceeds that of alkali metals which are regarded as major constituents.

Although the presence of a large quantity of the heavy metals in gallstones was pointed out long ago[40], most of the successive researches[18,41-45] have been limited merely to the detection of trace elements. The quantitative analysis of the heavy metals has not been made, what with the scantiness of samples and what with the incompleteness of the micro analytical method. Accordingly, reports on the quantitative analysis of heavy metals are very rare (copper was determined quantitatively by Newell[46] and Telfer[47], and lead, by Anderson[48]).

A gallstone was reported to be formed experimentally as a result of continuous feeding of heavy metals, such as copper[49,50], iron[51] and manganese[51], to animals, though physiological hindrances were caused by doing so. Yuta[18] reported that he ascertained the establishment of Hardy-Schulz's law on addition of various electrolytes to dialyzed bile, and also that, in this case, heavy metal ions, even if very small in quantity, took part in accerelating the coagulation of bile constituents by alkaline-earth metals. On the one hand these facts indicate the significance of heavy metals in the formation of gallstones. On the other hand Telfer[47] denies the importance of copper in cholelithiasis. However, the significance of heavy metals in a gallstone will be ascertained from their behavior in the process of the formation of a gallstone. Table I shows that the contents of these heavy metals decrease in order of mineral stone, cholesterinpigment-calcium stone and cholesterin stone. The average values of heavy metals of all samples of gallstones are arranged as follows:

Cu>Fe>Zn>Mn>Pb

However, there are some differences among the values of various groups of stones as follows:

Mineral stone.....
Cu>Fe>Mn>Zn>Pb
Cholesterinpigment-calcium stone.....
Cu>Zn>Fe>Mn>Pb
Cholesterin stone.....
Zn>Cu>Fe>Pb>Mn

When the content of each constituent in blood is taken as a standard, the ratios of the average contents in blood, bile and a gallstone are given in Table X (the figures shown in brackets indicate that the content of each of those constituents in bile is regarded as a standard).

TABLE X

	Blood	Bile	Gallstone
Fe	.....1.	0.052(1)	0.59(11)
Cu	.....1.	9.1 (1)	726. (80)
Zn	.....1.	1.2 (1)	24. (20)
Pb	.....1.	1.2 (1)	245. (203)
Mn	.....1.	3.6 (1)	327. (90)

That is, copper, zinc, lead and manganese are concentrated according to the process of the formation of a gallstone. A large quantity of iron is contained in blood as a constituent of hemoglobin, but its large proportion would be lost when hemoglobin decomposes to bilirubine and moves into bile.

The results of this study are insufficient to explain how these heavy metals take part in the formation of a gallstone, and for this purpose further studies should be made to reveal the content of these metals in the bile of cholelithiasic patients, and the status of presence of these metals in

39) U. Butturini and G. Meduri, *Giorn. Clin. Med.*, **31**, 693 (1950) : *C.A.*, **44**, 10304 (1950).
40) R. Schönheimer and W. Herkel, *Klinische Woch.*, **1931**, 345.
41) W. Gerlach, *Verhand. Deut. Pathol. Gesel.*, Tag. **27**, 277 (1934).
42) E. Müller, *Bioch. Z.*, **286**, 182 (1936).
43) E. Takino, *Trans. Soc. Pathol. Jap.*, **33**, 262 (1944).
44) E. Schairer, *Arch. Path. Anat. Physiol.*, **312**, 534 (1944) ; *C. A.*, **41**, 5953 (1947).
45) M. Mukai, *Medicine and Biology* (IGAKU-to-SEIBUTSUGAKU), **10**, 327 (1947).
46) W. E. Newell, *Brit. Med. J.*, **1**, 262 (1936) ; *C. A.*, **32**, 2602 (1938).
47) S. V. Telfer, *Glasgow Med. J.*, **27**, 181 (1946) : *C. A.*, **40**, 5825 (1946).
48) A. B. Anderson, *Biochem. J.*, **39**, 58 (1945).
49) T. Yokoyama, *J. Gastroenterology*, **10**, 708 (1935).
50) K. Fujita and T. Fukuda, ibid., **10**, 709 (1935).
51) K. Seki, ibid., **14**, 878, 921 (1939).

blood, bile and a gallstone (Epprecht et al.[22] ascertained the presence of copper calcium-bilirubinate in gallstone by means of X-ray analysis). But their significance can not be overlooked when the above result is considered together with those of pathological and colloidal studies. There is a positive correlation among the heavy metals at the 95% confidence interval, except the combination of iron and zinc.

(2) *Urinary Calculus and Urine.*—Studies of heavy metals in a urinary calculus have been undertaken mainly by means of qualitative spectrographic analyses[34,41,44,52-54]. Yokoyama[55] reported on the renal calculi alimentarily formed in animals by the use of copper and calcium. According to Ishibashi's report[56], zinc, lead, iron and tin in the crystallization of uric and oxalic acid were promoters. Yoshikawa[31] reported that greater quantities of copper, lead, tin, manganese and iron were contained in diseased urine than in normal urine.

The order of average values of heavy metal contents in urinary calculus and urine are arranged as follows:

Urinary calculus...Zn>Fe>Pb>Cu>Mn
Urine.....Fe>Zn>Cu>Pb>Mn

The concentrations of iron, copper, zinc, lead and manganese in urinary calculus are 114, 76, 365, 500 and 230 times, respectively, as much as those in urine. Zinc is the most abundant in urinary calculus just as copper is in a gallstone, and is considerably concentrated. These facts suggest the similar function of copper and zinc in the formation of gallstone and urinary calculus, respectively. Parsons[57] reported that $Zn_3(PO_4)_2 \cdot 4H_2O$ was found in urinary calculus by means of X-ray analysis. Although the content of lead in a urinary calculus or a gallstone is not always abundant compared with the other constituents, the rate of its concentration is the highest both in cases of urinary calculus (from urine) and gallstone (from bile). In a living body, lead is usually accumulated in the bone in which calcium salts are more abundant than in any other parts of the body. These results indicate the strict connection between lead and the formation of calculi which mainly consist of calcium salt, as suggested by Gerlach[41].

There are positive correlations between manganese and copper and between lead and zinc in a urinary calculus at the 95% confidence interval.

(3) *Pancreatic Calculus.*—Two reports were made by Schairer[44] and by Kobayashi et al.[58] in regard to the spectrographic analysis of the pancreatic calculus, but the determination of heavy metal constituents in them has not been made as yet.

Table VI indicates that a relatively greater quantity of contents of zinc and lead exists and that iron, copper and manganese are contained but in a very small quantity. Especially, zinc is the most abundant of all, and it is a matter of interest that this relates to the fact that *Insulin*[59]—which is well known as an organic compound that contains zinc in it—exists in the pancreas.

Both of the samples were composed mainly of calcium carbonate, while zinc is contained in *Carbonic anhydrase*[60] as a constituent, and these facts suggest the existence of some relation between zinc and the formation of a pancreatic calculus.

3. Distribution of Inorganic Constituents between the Internal and the External Portion of a Gallstone.—From the standpoint of mechanical structure, gallstones are classified into three types, radial, layer and a combination of the two.

Table XI shows the distribution of inorganic constituents in the internal and the external portions of three samples of gallstones which are typical ones having layer structures. The sample G-4011 consists of mineral stone (internal portion) and cholesterinpigment-calcium stone (external portion). Both the internal and the external portion of samples G-5019 and G-8004 consist of mineral stone and cholesterinpigment-calcium stone respectively. These results indicate, with a few exceptions in the case of calcium and phosphorus, that the internal portion is richer in inorganic constituents, especially heavy metals, than the external portion.

These layer structures demonstrate that the sedimentation circumstance is changed

52) C. P. Mathe and R. C. Archambeault, *Pacific Coast Med.*, **7**, 13 (1940); *C. A.*, **36**, 5538 (1942).
53) T. Sekimura, *Jap. J. Urol.*, **36**, 252 (1944).
54) M. M. Kovalev, *Klin. Med.*, **33**, No. 11, 54 (1955); *C. A.*, **50**, 7290 (1956).
55) T. Yokoyama, *J. Jap. Surg. Soc.*, **38**, 722 (1937).
56) M. Ishibashi, *J. Kurume Med. Assoc.*, **13**, 39 (1950).
57) J. Persons, *Science*, **118**, 217 (1953).
58) H. Kobayashi and Y. Yasumoto, *Medicine and Biology* (IGAKU-to-SEIBUTSUGAKU), **13**, 283 (1948).
59) A. M. Fischer and D. A. Scott, *Biochem. J.*, **29**, 1048 (1935).
60) D. Keilin and T. Mann, ibid., **34**, 1163 (1940).

TABLE XI

DISTRIBUTION OF INORGANIC CONSTITUENTS BETWEEN THE INTERNAL AND THE EXTERNAL PORTION OF HUMAN GALLSTONES

Sample		Ash, %	Ca mg./g.	Mg mg./g.	P mg./g.	Fe p.p.m.	Cu p.p.m.	Zn p.p.m.	Pb p.p.m.	Mn p.p.m.
G-4011	Internal portion	12.57	31.82	4.37	21.30	2,290	10,400	385	84	277
	External portion	6.95	34.92	3.27	4.77	61	281	116	46	33
G-5019	Internal portion	9.08	45.84	3.90	5.28	373	2,440	574	95	118
	External portion	8.26	35.31	3.71	9.32	68	1,382	279	43	83
G-8004	Internal portion	2.95	14.90	0.87	1.78	277	858	451	148	94
	External portion	1.33	5.37	0.42	1.82	71	255	149	51	55

at the time of the formation of a gallstone, and especially that the character of the internal portion has a direct connection with the reason why a calculus is formed. Although it is not easy to draw out a conclusion from the results of only three examples, from the above mentioned standpoint, it is interesting that the inorganic constituents, especially heavy metals, are abundant in the internal portion of a gallstone.

Summary

Quantitative analyses of major constituents (calcium, magnesium, phosphorus, sodium and potassium) and heavy metal constituents (iron, copper, zinc, lead and manganese) were made on human stones (gallstone, urinary and pancreatic calculus) and their respective humours (blood, bile and urine). Then the distribution of these elements and the mutual relations present among them were discussed.

(1) According to the process of formation of gallstone (blood→bile→gallstone), the concentration of calcium, magnesium and phosphorus increases, while that of sodium and potassium decreases in case of major constituents, On the other hand, the concentration of heavy metal constituents, copper, zinc, lead and manganese increases. The concentration of iron decreases in the process of blood→bile and then increases again in the process of bile →a gallstone, whilst copper is extremely concentrated throughout these processes.

(2) In the process of urine→urinary calculus, each of major constituents increases; calcium, magnesium and phosphorus are extraordinarily concentrated, and heavy metal constituents also increase zinc is especially the most abundant of all. The correlation between lead and the calcification phenomenon in biological materials is observed, for lead indicates the highest concentration ratio in every case of gallstones (203 times that of bile) and urinary calculi (500 times that of urine).

(3) Pancreatic calculi differ greatly from the other stones, as their major constituents are calcium carbonate and they contain but little of the other constituents. Among heavy metals, zinc and lead are contained in a relatively high quantity; it seems that special connections exist between zinc and *Insulin* and between zinc and *Carbonic anhydrase*.

(4) The internal portion of a gallstone that is of layer structure contains a greater quantity of heavy metals than the external portion.

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Studies on the Structure of Bisacetylacetone-Nickel(II) by the X-ray Powder Method

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Pauling[1] proposed the tetrahedral configuration of bisacetylacetone-nickel(II) on the basis of the paramagnetic property of the complex with the aid of his theory. On the other hand, experiments[2,3] by electron diffraction of gas gave the result that the molecule assumes a planar configuration. If it is noted for this contradiction that the electron diffraction study dealt with free molecules at high temperature (about 170°), whereas the magnetic measurement was made on the solid at room temperature, it seems likely that the configuration of the molecule depends on the environment of a molecule*1.

According to the recent theoretical consideration[4], however, the magnetic data (paramagnetic) are not necessarily conclusive for the choice to be made between the tetrahedral and the planar arrangement. Therefore the X-ray method has been undertaken to afford a more direct evidence for the configuration of a bisacetylacetone-nickel(II) molecule in the solid state at room and high temperatures. Although X-ray analysis on single crystals is desirable for the complete determination of the structure of the molecule, it was difficult to prepare single crystals of an appropriate size of an anhydrous nickel complex. Therefore, investigations were carried out by the powder method. The powder patterns of nickel complex were compared with those of copper and zinc complexes.

Experiments and Results

The powder photographs*2 of copper, nickel and zinc complexes were prepared by the use of Cu-Kα radiation from the samples enclosed in a capillary tube made of glass. Samples recrystallized from ethanol were furnished by Dr. Sone. Anhydrous compounds were prepared by sublimation in vacuo; hydrates*3 were obtained by leaving them to stand in moist air. The patterns thus obtained are shown in Fig. 1.

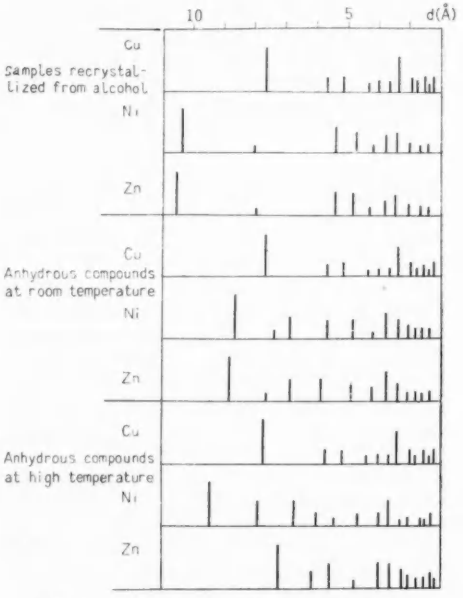

Fig. 1. The powder patterns of Cu, Ni and Zn acetylacetones.

1) Bisacetylacetone-copper(II). The sample recrystallized from ethanol and that sublimed in vacuo, both of which were dark blue in color, gave identical powder patterns. The sample heated up to about 200° also gave the same powder patterns.

2) Bisacetylacetone-nickel(II). The sample recrystallized from ethanol and the hydrate

1) L. Pauling, "Nature of the Chemical Bond", Cornell Univ. Press, Ithaca, (1948), p. 119.
2) S. Shibata, M. Kishita and M. Kubo, *Nature*, **179**, 320 (1957).
3) S. Shibata, This Bulletin, to be published shortly.
*1 From this point, magnetic measurements have been made on the solid at high temperature, but the results showed no indication of the change in magnetic moment with temperature. (Ref. 2).
4) D. P. Craig, A. Maccoll, R. S. Nyholm, L. E. Orgel and L. E. Sutton, *J. Chem. Soc.*, **1954**, 332: Y. Kuroda and K. Ito, *J. Chem. Soc. Japan, Pure Chem Sect.*, **76**, 762 (1954).

*2 The patterns of cobalt(II) complexes were also taken by the use of Fe-Kα radiation. The results showed that pink hydrate and red-violet anhydrate were isomorphous with the corresponding nickel(II) complexes, respectively.
*3 Only the copper(II) complex was not hydrated and did not contain alcohol when it was recrystallized from an alcoholic solution.

thereof*4, both of which were blue in color, gave the identical patterns. On heating at about 130° the green color of the anhydrous compound changed into yellow-green, which color persisted up to about 170°. These two samples showed different powder patterns as shown in Fig. 1, suggesting that a change was taking place in the crystal structure. They are designated as the room and the high temperature modification, respectively. The green anhydrous compound (the room temperature modification) was readily hydrated in moist air, but the yellow-green anhydrous compound (the high temperature modification) was fairly stable even in moist air. The blue hydrate*5 was reversely converted into a green anhydrous compound at about 80°.

TABLE I

INTERPLANAR SPACINGS FROM POWDER PATTERNS*

$Cu(C_5H_7O_2)_2$		$Ni(C_5H_7O_2)_2$		$Zn(C_5H_7O_2)_2$	
d(Å)	I	d(Å)	I	d(Å)	I
7.61	vs	8.66	vs	8.82	vs
5.66	w	7.41	vw	7.60	vw
5.14	w	6.82	mw	6.85	m
4.35	vw	5.67	mw	5.86	mw
3.99	w	4.80	mw	4.88	mw
3.60	w	4.18	vw	4.22	w
3.37	ms	3.78	m	3.73	m
2.94	w	3.39	mw	3.40	mw
2.78	vw	3.07	w	3.09	w
2.50	w	2.83	w	2.82	w
2.34	vw	2.61	w	2.63	vw
2.22	w	2.38	w	2.35	w
2.00	w				

* The lines which may be due to an impurity such as a hydrate were not listed.

3) Bisacetylacetone-zinc(II). White bisacetylacetone-zinc(II) gave three different patterns i. e., one for the hydrate, which was found to be isomorphous with the hydrate of nickel complex, and two for the anhydrous compound. This behavior with changing temperature was quite similar to the case of bisacetylacetone-nickel(II).

Discussion

The crystal structure of bisacetylacetone-copper(II) has already been determined by Koyama et al.[5] by X-ray analysis on a single crystal. The result showed that the complex takes a planar (square) configuration*6; a copper atom completes a distorted octahedron. On the other hand, the tetrahedral nature of four-covalent zinc(II) complexes is established from theoretical and experimental[6] basis. When the powder patterns of anhydrous copper, nickel and zinc complexes are compared with one another, the patterns of the room temperature modification of the nickel complex are clearly distinguishable from those of the copper complex, while it is essentially identical with those of the zinc complex. The interplanar spacings are listed in Table 1. Accordingly, it seems to be sure that the bisacetylacetone-nickel(II) has a tetrahedral configuration rather than a planar one, in agreement with the deduction by Pauling.

In order to investigate a possible change in the structure of bisacetylacetone-nickel (II) with temperature, the powder patterns of anhydrous copper, nickel and zinc complexes were taken at high temperature, but these patterns were different from one another. The pattern of high temperature modification of the anhydrous nickel complex seems to show a possibility of the change in the molecular structure of this complex, but does not simply permit a choice between a tetrahedral and planar coordination.

Recently Bullen[7] investigated the crystal structure of anhydrous bisacetylacetone-nickel(II) by X-ray analysis on a single crystal, determined the lattice constants, and reported the presence of trinuclear molecules in the crystal. Although he did not definitely elucidate the molecular structure of the complex, the short distance between nickel atoms would favor the planar configuration of the complex. On the other hand, it was found that the interplanar spacings calculated by the use of his lattice constants are more consistent with those of the high temperature modification than with those of room temperature modification of this complex. Therefore, it is possible that the nickel complex of the high temperature modification may have the planar configuration, although the determination is not complete. If this

*4 Experiments showed that the hydrate contains two molecules of water per one molecule of the complex.

*5 It seems likely that, in the hydrate, water molecules are loosely coordinated to metal atoms and presumably from an octahedral configuration about the central metal atom with other ligand atoms and are easily removed on drying at high temperature.

5) H. Koyama, Y. Saito and H. Kuroya, *Journal of the Institute of Polytechnics, Osaka City University*, **4**, Series C, 43, (1953).

*6 The square planar configuration of a free molecule of bisacetylacetone-copper(II) has been indicated also by electron diffraction investigation. (S. Shibata and K. Sone, This Bulletin, **29**, 852 (1956)).

6) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds", Prentice-Hall, New York, (1952), p. 245. J. C. I. Liu and J. C. Bailar, *J. Am. Chem. Soc.*, **73**, 5432 (1951); H. Koyama and Y. Saito, This Bulletin, **27**, 113 (1954).

7) G. J. Bullen, *Nature*, **117**, 537 (1956).

is the case, this fact indicates that the configuration of bisacetylacetone-nickel(II) varies with temperature.

Conclusion

X-ray powder pattern method led to the result that four-covalent bisacetylacetone-nickel(II) takes a tetrahedral configuration in agreement with the presumption of Pauling. On the other hand, a previous experiment by electron diffraction showed that the free molecule of this complex has a planar configuration. Thus, these experimental evidences lead to the following interesting inference: the configuration of anhydrous bisacetylacetone-nickel(II) varies with the change of the phase from gas to solid (or with temperature). This is evidently due to the fact that the energy difference between the tetrahedral and planar configuration is quite small.

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Hydrolysis of Carbohydrates in the Presence of Hydrochloric Acid or Acidic Ion-exchange Resins

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There have been several reports concerning the effect of the size of ion-exchange resin catalyst on the rate of organic reactions; e. g., esterification of oleic acid[1], hydrolyses of sucrose[2] and lower esters[3], and iodine-acetone reaction[4]. None of these investigators, however, has given any general quantitative conclusion theoretically supported. The present study is concerned with the development of this subject. First, the result of the hydrolysis of potato amylose and sucrose is shown in this paper. The amylose was chosen because of its high molecular weight and sucrose because of its low molecular weight.

Experimental

Preparation of Amylose.—A modified procedure[5] of K. H. Meyer's method was employed. That is, the amylose in potato starch was extracted into hot water (70°C), precipitated with *n*-butanol, dissolved once more into hot water (60°C), reprecipitated with ethanol and then dried at 70°C to a constant weight. The yield from 250 g. starch was 15 g.

Molecular Weight and Purity of Amylose.—The number average degree of polymerization of the amylose was measured by K. H. Meyer's method[6] (3,5-dinitrosalicylic acid method) and found to be 175 ± 10. Hence the number average molecular weight was $(3.2\pm0.2)\times10^4$. The absorption maximum of the amylose-iodine complex, prepared according to R. M. Mc Cready's method[7], was 625 mμ, which agreed well with R. E. Rundle's[8] result (626 mμ, for potato amylose). Hence the purity of the amylose was high enough to carry out this work.

Sucrose.—Twenty % aqueous solution of commercial crystalline sugar, demineralized by means of ion-exchanger, was used.

Ion-Exchange Resins.—Strongly acidic ion-exchange resin Amberlite IR-120, conditioned and washed, was fractionated into ten portions according to the grain size. The six larger fractions were prepared by a combined process of sieving and sedimentation of the original spherical resins. The four smaller fractions were

1) C. L. Levesque and A. M. Craig, *Ind. Eng. Chem.*, **40**, 96 (1948).
2) G. Bodamer and R. Kunin, ibid., **43**, 1082 (1951).
3) L. P. Hammett, et al., *J. Am. Chem. Soc.*, **71**, 1284 (1949), ibid., **75**, 1788, 5834 (1953).
4) S. L. Bafna, *J. Phys. Chem.*, **59**, 1199 (1955).
5) E. Husemann und R. Resz, *J. Polymer Sci.*, **19**, 397 (1956).
6) K. H. Meyer, *J. Phys. Colloid Chem.*, **53**, 319 (1949); J. Nikuni, *Starch Chemistry (in Japanese)*, Asakura-shoten Ltd., Tokyo, Japan (1951), p. 359.
7) R. M. McCready and W. Z. Hassid, *J. Am. Chem. Soc.*, **65**, 1154 (1943).
8) R. E. Rundle, ibid., **66**, 111 (1944).

prepared by sedimentation of pulverized resins. The average radius (R) of each fraction was determined microscopically according to the formula (1),

$$R=\bar{d}/2=\sum nd^3/2\sum nd^2 \quad (1)$$

where d is the horizontal diameter of each particle, n is the number of particles, and $\bar{d}$ is the average diameter. The values of R were found to be 482, 418, 332, 300, 219, 191, 56.9, 37.2, 26.8 and 17.5 μ respectively.

Hydrolysis of Amylose (hydrochloric acid catalyst). — Two % amylose solution in N-sodium hydroxide was acidified with hydrochloric acid and stirred in a sealed vessel at 65±0.1°C. After every five minutes, a portion of the reaction mixture was taken out and neutralized with alkali to stop the reaction. This neutral solution was used for both preparation of the starch-iodine complex and determination of the number average degree of polymerization (P_n). The starch-iodine complex was prepared by the Mc Cready's method[7]. P_n was determined according to the Meyer's method[6], using glucose as a standard.

Hydrolysis of Amylose (pulverized resin catalyst) — Two % amylose solution in N-sodium hydroxide was demineralized with ion-exchange resins, added to a known volume of resin suspension containing a known weight of pulverized resin, and then stirred at 65±0.1°C. After a given period, the reaction mixture was quickly filtered to remove the resin. The filtrate was used for both preparation of starch-iodine complex and determination of P_n.

Hydrolysis of Sucrose.—The demineralized 20% sucrose solution was mixed with catalyst (conc. hydrochloric acid, pulverized resin suspension, or swollen spherical resin filtered to remove the extra water) and stirred in a sealed vessel at 50±0.1°C. The stirring velocity was kept within a range of 500—550 r. p. m. and did not affect the reaction rate. After a given period, a portion of the reaction mixture was taken out to measure the optical rotation (α) by a saccharimeter. The α value at complete hydrolysis was also measured (α_∞). When $\ln(\alpha-\alpha_\infty)$ were plotted against t (reaction time), straight lines were obtained, the slopes indicating the rate constants k according to the formula (2).

$$kt=\ln(\alpha-\alpha_\infty)+const. \quad (2)$$

Results and Discussions

Hydrolysis of Amylose

i. N-Hydrochloric acid catalyst—Amylose was easily hydrolysed at 65°C. Both P_n of the hydrolyzate (Table I) and the absorption spectrum of the hydrolyzate-iodine complex (Fig. 1) changed with time. The relation between the wave length of the absorption maximum and P_n was nearly in harmony with that in the case of synthetic dextrins worked by Swanson[9] (Table II), showing that the mechanism of hydrolysis was just the inverse of synthesis. The mean rate constants through time 0~t (k in Table II) were computed, applying Ekenstam's[10] equation (3) of random degradation.

$$\ln[1-P_n(t)^{-1}]=\ln[1-P_n(0)^{-1}]-kt \quad (3)$$

k increased as the reaction proceeded, resembling Freudenberg's[11] result of hydrolysis of soluble starch in 50% sulfuric acid.

TABLE I

HYDROLYSIS OF AMYLOSE (HCl CAT.)

Time (min.)	$-\log T$[a]	P_n	$k\times10^4$ (min.$^{-1}$)
0	0.010	175±10	—
5	0.062	95± 3	4.3±0.5
10	0.144	55± 1	5.5±0.3
15	0.170	49± 1	4.3±0.2
20	0.303	31.2±0.3	5.9±0.2
25	0.355	27.0±0.2	6.0±0.1
30	0.450	21.9±0.1	6.0±0.1
35	0.575	17.5±0.1	6.6±0.1

a) T is the transmission coeff. of sample soln. treated with 3, 5-dinitrosalicylic acid, at the wave length of 525 mμ. Sugar concentration was 1.67 % concerning the original amylose.

TABLE II

ABSORPTION MAXIMA OF STARCH-IODINE COMPLEXES

P_n	Wave length (mμ) D[a]	A[a]
7.4	500	
12.9	520	
17.5±0.1		550
18.3	540	
20.2	560	
27.0±0.2		560
29.3	580	
34.7	580	
49±1		585
55.6	600	
95±3		600
97.4	600	
146.5	600	
175±10		625

a) D: Synthetic dextrins by Swanson[9].
A: Hydrolyzed amyloses by the author.

9) M. A. Swanson, *J. Biol. Chem.*, **172**, 825 (1948); J. Nikuni, *Starch Cecmistry*, Asakura-shoten Ltd., Tokyo, Japan (1951), p. 57.
10) A. af Ekenstam, *Ber.*, **69**, 540, 553 (1936).
11) K. Freudenberg, et al., *Ber.*, **63**, 1519 (1930).

TABLE III
HYDROLYSIS OF AMYLOSE (RESIN CAT.)

Resin	Time (min.)	Ppt.[c]	$-\log T$
$R=17.5\mu$[a]	0	—	0.002
	30	—	0.004
	60	—	0.003
	94	—	0.005
	124	+	—
original[b], unfractionated	0	—	0.004
	10	—	0.004
	30	—	0.004
	40	+	—

a), b): Sugar concentrations of the sample solutions were 1.33% and 2.00%, respectively.
c) Ppt. of retrograded amylose.

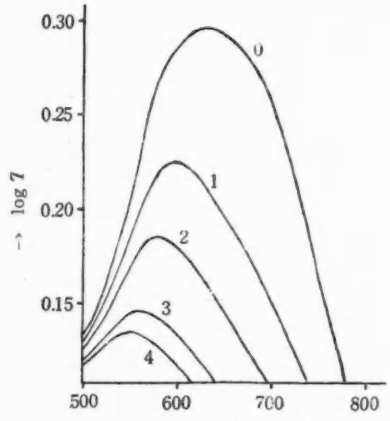

Fig. 1. Absorption spectra of iodine-hydrolyzed amylose complexes.

No.	0	1	2	3	4
Reaction time (min.)	0	5	15	25	35

ii. resin catalyst. — The resin of the smallest size ($R=17.5\,\mu$) was used at a concentration of nearly 0.02 equivalent per liter and caused no significant change in either the transmission coefficient of solution for measurement of P_R (Table III) or the absorption maximum of tho iodine complex. There seemed to be, therefore, no significant hydrolysis even at the end of 94 min. at 65°C. Several other experiments, using resins of a larger size at concentrations up to one equivalent per liter, also showed no proof of hydrolysis. One of these data is also shown in Table III.

iii. Comparison of hydrochloric acid and resin catalyst.—These experimental results show that the efficiency of resin catalyst is negligibly small as compared with hydrochloric acid in the case of hydrolysis of amylose. It is more desirable to compare their efficiencies numerically. But unfortunately it is impossible in this study.

Hydrolysis of Sucrose. — Sucrose was easily hydrolyzed by means of resin catalysts at 50°C and the first order rate constants (k) were obtained. The second order rate constants (k') were computed according to the formula (4),

$$k'=k/\rho H=kV/A \qquad (4)$$

where ρ is the volume ratio of swollen resin and the outer solution, H the exchange capacity per unit volume of the resin, V the total volume of the solution, and A the total exchange capacity of the resin catalyst.

The relationships $k'\sim R$ and $k'\sim 1/R$ are shown in Fig. 2. If the rate were proportional to the surface area of the catalyst, $k'\sim 1/R$ curve would be a straight line passing through the original point. Though the curve in Fig. 2 does not agree with this, it is inclined to approach a linear figure near the original point ($R\to\infty$). On the other hand, if the rate were proportional to the volume of resin phase, $k'\sim 1/R$ curve would be a straight line parallel to the $1/R$ axis. The curve in Fig. 2 does not agree with this, either. But it seems probable that the curve approaches a linear horizontal line as R approaches zero.

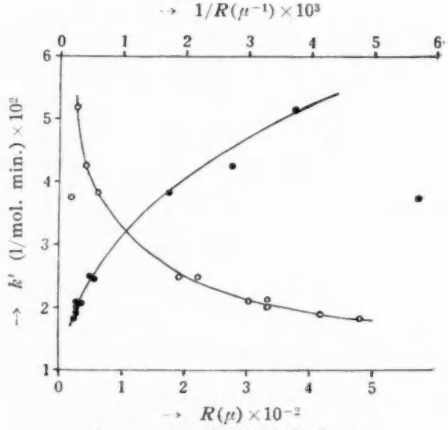

Fig. 2. $k'\sim R$, $k'\sim 1/R$ Curves.

At any rate, R and k' are related in a mediate way between the afore-mentioned two extremes. It is concluded that neither the surface only nor the whole resin phase uniformly acts as catalyst, but the real is

a compromise. This is a proof that the overall rate is largely affected by the diffusion rate of sucrose in the resin. This knowledge will be valuable in studying the theoretical expression of the overall rate.

In the case of hydrochloric acid catalyst, k was directly proportional to the concentration of the acid. The proportionality constant (equals k', because $\rho=1$) was found to be 0.104 l/mol. min. at 50°C, which was greater than k' of any resin catalyst.

Summary

Potato amylose and sucrose were hydrolyzed in the presence of hydrochloric acid or acidic ion-exchange resin catalyst. In hydrolysis of amylose at 65°C, the efficiency of resin catalysts was negligibly small as compared with that of hydrochloric acid. In hydrolysis of sucrose at 50°C, the efficiency of resin catalysts was less than that of hydrochloric acid and different according to their sizes. The relation between the average radius and the rate constant was obtained in a broader range than ever.

This work was supported by a grant-in-aid for Development of Scientific Research (1956) from the Ministry of Education. The author would like to thank Professor I. Kagawa for his encouragement. Thanks are also due Mr. T. Kainuma for his aid in carrying out the experiment.

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Spectrophotometric Method for the Determination of Small Amounts of Sulfate Ions

By Iwaji IWASAKI, Satori UTSUMI, Ken HAGINO, Toshikazu TARUTANI and Takejiro OZAWA

(Received February 28, 1957)

A more rapid and accurate method for determining sulfate of low concentration in natural waters is desirable. An indirect colorimetric determination of sulfate, using solid barium chromate and diphenylcarbazide was previously reported by us[1]. However, this method can only be applied to the determination in the range 0.2 to 10 p. p. m. Recently, T. Kato, Y. Nomizo and K. Shinra[2] studied a photometric method of determination of 2 to 120 p. p. m. of sulfate using a perchloric acid solution of barium chromate and ammonia. The authors have devised an improved method for the determination of sulfate over the range 0.3 to 100 p. p. m., using barium chromate suspended in an acid solution.

Experimental Results and Discussion

Reagents

(1) *Standard solution of sulfate* (1,000 p. p. m. SO_4^{2-}): —Recrystallized potassium sulfate (guaranteed reagent) 1.815 g. is dissolved and diluted to 1 l. with distilled water. Solutions of various concentrations can be prepared by diluting this stock solution. All solutions are stored in polyethylene bottles.

(2) *Barium chromate suspension.*—One hundred cc. of a hot solution of barium chloride containing 10 g. of $BaCl_2$-$2H_2O$ is added slowly to 800 cc. of a hot solution of potassium chromate containing 8 g. of K_2CrO_4 to obtain a yellow precipitate of barium chromate. This precipitate is washed three times by decantation with about 500 cc. portions of hot water, dissolved in 100 cc. of hot 2 M hydrochloric acid, and diluted to 700 cc. with hot water. A 105~110 cc. portion of 2 M ammonia water is added slowly in order to reprecipitate the barium chromate. The precipitate thus obtained is washed again in the same way as mentioned above, and after drying at 105°C for about one hour, it is pulverized in an agate motar. Barium chromate suspension is prepared by adding 2.5 g. of the barium chromate in 200 cc. of the aqueous solution consisting of 0.5 M acetic acid and 0.01 M hydrochloric acid. The pH of this suspension is about 2.4. The reagent is stored in a polyethylene bottle.

(3) *Ammonia water containing calcium ions.*—

1) I. Iwasaki, S. Utsumi and T. Tarutani, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **74**, 400 (1953).
2) T. Kato, Y. Nomizo and K. Shinra, ibib., **76**, 373 (1955).

The reagent is prepared by dissolving 1.85 g. of $CaCl_2 \cdot 2H_2O$ in 500 cc. of purified 6 M ammonia water (1 mg. Ca^{2+}/cc.). The solution is stored in a container shown in Fig. 1.

(4) *Diphenylcarbazide reagent.*—The reagent is prepared by dissolving 1 g. of diphenylcarbazide in a mixture of 100 cc. of ethanol and 1 cc. of 1 M hydrochloric acid. This reagent is stable for 2~3 weeks in a brown bottle.

(5) Ethanol (guaranteed reagent) is used after being purified by distillation.

(6) Hydrochloric acid (6 M).

Apparatus

(1) Measurements were carried out with the Hitachi Type E. P. U. 2 spectrophotometer and with 5 mm. absorption cells.

(2) Centrifuge (about 4,000 r. p. m.).

(3) Glass filter (15 cc., No. 4),

(4) Centrifuge tube (15 cc.) with a glass stopper[3].

(5) Apparatus for storing ammonia water. To keep ammonia water free from carbon dioxide in the air, a polyethylene bottle attached with a buret and two soda-lime tubes as shown in Fig. 1 was used.

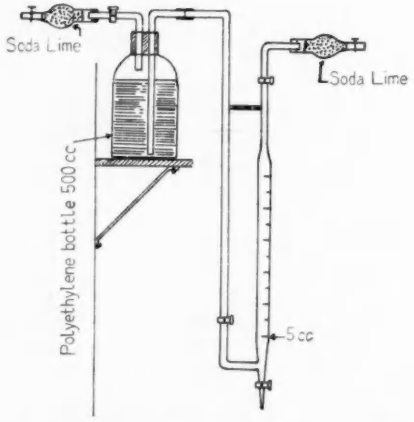


Fig. 1. Apparatus for storing ammonia water.

Procedure

Pipet 5 cc. of a sample solution into a centrifuge tube, add 2 cc. of a uniformly suspended solution of barium chromate and mix. After standing for 2~3 minutes, add 0.5 cc. of ammonia water containing calcium ions and mix. Add 5 cc. of ethanol and shake for about one minute (pH of the solution is about 9.8). Let the mixture stand for about ten minutes.

When the sulfate concentration of a sample solution is higher than 20 p. p. m., measure the absorbance of the supernatant solution at 370 mμ directly after the centrifugation (Procedure A).

For the cases of concentration lower than 20 p. p. m., filter the supernatant solution of the centrifugate gradually through a glass filter, add 1 cc. of diphenylcarbazide reagent and 0.7 cc. of 6 N hydrochloric acid to the filtrate and mix well. After five minutes, measure the absorbance of this violet colored solution at 545 mμ (Procedure B).

Absorption Spectral Curves

The absorption of the yellow color due to the chromate ion replaced in the solution was measured over the wave length range 230 to 450 mμ. The absorption spectral curve is shown in Fig. 2, in which there are two absorption maxima at about 275 mμ and 370 mμ.

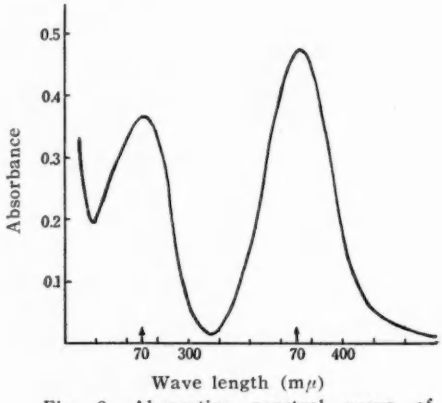


Fig. 2. Absorption spectral curve of chromate.

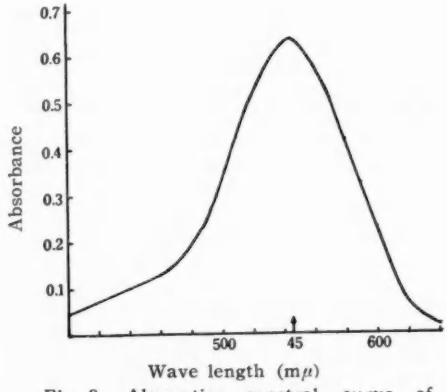


Fig. 3. Absorption spectral curve of Cr(VI)-Diphenylcarbazide.

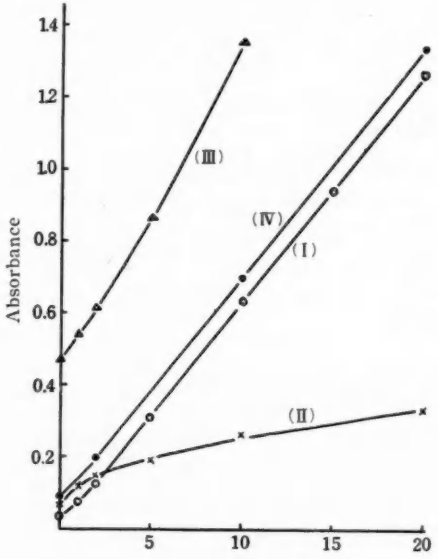
The chromate ion reacted with diphenylcarbazide in an acidified solution to give a violet coloration. The absorption of the violet color was measured over the range 400 to 640 mμ, as shown in Fig. 3, in which there is one absorption maximum at about 545 mμ.

3) S. Utsumi, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **74**, 35 (1953).

The maximum color development of the solution(chromium(VI)-diphenylcarbazide) was obtained after letting it stand for five minutes at room temperature. The violet color was stable for a long time.

Calibration Curves

(1) *For concentrations lower than 20 p. p. m. of sulfate.* — The calibration curve(I) shown in Fig. 4 was obtained by Procedure B with standard sulfate solutions of known concentration. The absorbance and the concentration were in a good linear relationship for the range 2 to 20 p. p. m. SO_4^{2-}, but were not good for concentrations lower than 2 p. p. m.



Concentration of Sulfate p. p. m.

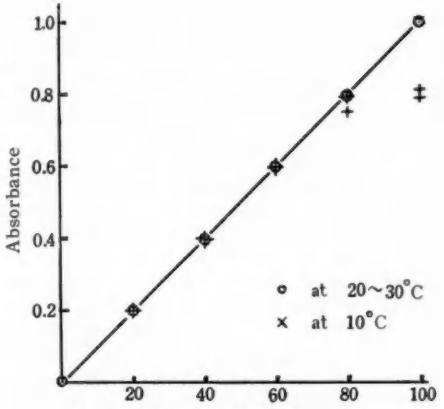
Fig. 4. Calibration curves at 545 mμ, 5 mm cells.

The calibration curve had to be renewed whenever solid barium chromate was newly prepared. In order to obtain reproducible results, it was necessary to use barium chromate suspension which had been stored for a few days after preparation.

When a blank test was made with redistilled water, a faint coloration appeared. The absorbance of the blank test solution was small and constant. The lowest concentration of sulfate, whose coloration could be distinguished from that of the blank test was 0.3 p. p. m. SO_4^{2-} (3.1×10^{-6} M/l).

The calibration curve(I) shown in Fig. 4 was reproducible within the error of ±0.2 p. p. m. SO_4^{2-}. Thus, the method may be available for determining sulfate over the range from 0.3 to 20 p. p. m.

(2) *For concentrations higher than 20 p. p. m.* SO_4^{2-}.—The calibration curve shown in Fig. 5 was obtained by Procedure A using standard sulfate solutions.



Concentration of Sulfate p. p. m.

Fig. 5. Calibration curve at 370 mμ, 5 mm cells.

As can be seen in Fig. 5, Beer's law was conformable up to 100 p. p. m. and 70—80 p. p. m. of sulfate respectively at 20—30°C and 10°C. Since barium chromate was more soluble at higher temperatures and hence a sufficient amount of barium ion was available for the precipitation of barium sulfate, it seemed that sulfate of higher concentrations could be determined at higher temperatures.

The absorbance of the blank solution was negligible. The calibration curve was reproducible within 1%.

Conditions for the Analysis

(1) *Reasons for the use of barium chromate suspension.*—When a dilute solution of barium chromate in acidic medium was used instead of its suspension, there was a tendency to form a colloidal solution after the addition of ammonia water. Moreover, the calibration curve was not reproducible. For an accurate determination of a small amount of sulfate, the use of barium chromate suspension was better than that of its solution, because particles of barium chromate in the suspension carried colloidal precipitates of barium

chromate, formed by the addition of ammonia water.

(2) *Influence of acids in barium chromate suspension on the determination of sulfate.* —When a barium chromate suspended in distilled water was used in Procedure B, instead of the suspension mentioned above, the calibration curve(II) as shown in Fig. 4 was obtained, which was not linear with respect to the concentration. When a barium chromate suspended in 0.5 M acetic acid was used, sulfate ions could be determined only up to the concentration of 40—50 p. p. m. at 20—30°C (Procedure A).

On the other hand, when a barium chromate suspended in 0.01 M hydrochloric acid was used, the clear solution, once obtained after the addition of ammonia water containing calcium ion followed by centrifugation, gradually became turbid owing to the formation of calcium carbonate with carbon dioxide in the air.

(3) *Effect of the amounts of barium chromate.*—It was found that the coincident results were obtained by the use of between 2 to 3 g. of reprecipitated barium chromate in 200 cc. of the acid solution (0.5 M acetic and 0.01 M hydrochloric acid). Therefore, the authors would like to recommend the use of 2.5 g. of barium chromate.

(4) *Effect of alcohol.* — Since barium chromate was much less soluble in ethyl alcohol than in water, the influence of ethanol upon the determination of sulfate was examined. The experiment was carried out under the following conditions: 10 cc. of a sample solution was pipetted into a centrifuge tube, then 2 cc. of barium chromate suspension was added and mixed. After being set aside for 2—3 minutes, 0.5 cc. of ammonia water containing calcium ions was added and shaken for about one minute without ethanol. The subsequent treatment was done in the same way as procedure B, by which the calibration curve(III) in Fig. 4 was obtained. Not only was the absorbance of the blank solution larger owing to the greater solubility of barium chromate, but the calibration curve did not give a linear relationship, bending upward though slightly.

In order to decrease the solubility of barium chromate, 5 cc. of ethanol was used for 5 cc. of the sample solution.

(5) *Effect of the calcium ion in ammonia water.*—Since barium carbonate was also difficultly soluble, the absorbance would be considerably affected by the presence of carbonate in ammonia water. Without calcium ions, calibration curve(IV) shown in Fig. 4 was obtained. The curve was influenced by the concentration of carbonate in ammonia water. In order to remove the dissolved carbonate, the authors recommend the addition of some calcium ions into the ammonia water.

(6) *Effect of temperature.* — Since the concentration range of determination was affected by temperature, as shown in Fig. 5, it was desirable to carry out Procedure A at 20—30°C in order to determine the sulfate up to 100 p. p. m. satisfactorily. On the other hand, in the case of Procedure B the effect of temperature was negligible.

Influence of Various Ions.

The effect of different kinds of ion on this method was examined in the presence of 10 p. p. m. SO_4^{2-} and in its absence. The experimental results were summarized as follows.

(1) Neither sodium nor chloride ions interfere even when present in the amount of 5,000 p. p. m.

(2) One thousand p. p. m. of potassium or ammonium ions do not interfere, but 5,000 p. p. m. of each ion gives lower results.

(3) One thousand p. p. m. of perchlorate and 50 p. p. m. of silicate ions have no effect.

(4) The presence of nitrate ions gives somewhat higher results at 50 p. p. m. and considerably higher results at 1,000 p.p.m.

(5) The presence of carbonate or bicarbonate does not interfere at 50 p.p.m. but at 100 p. p. m. slightly higher results are obtained. When larger amounts of these substances are present, it is necessary to remove them beforehand. Carbonate (or bicarbonate) can easily be expelled by boiling a sample solution for a short time after neutralization with dilute hydrochloric acid. The necessary amount of hydrochloric acid may be determined by neutralizing an aliquot portion of the same sample, using methyl orange as indicator.

(6) Neither 100 p. p. m. of magnesium or calcium ions, nor 10 p. p. m. of strontium ion interfere.

(7) Each 10 p. p. m. of Ag^+, Zn^{2+}, Hg^{2+}, Fe^{3+}, Al^{3+} or Mn^{2+} ions does not interfere.

(8) Since lead chromate is difficultly soluble, lead ions interfere even in small amounts.

(9) As the copper ion also reacts with diphenylcarbazide, the presence of copper is undesirable. When the copper ion is present in a sample solution, 0.1 cc. of E·

D. T. A. (0.2 M) should be added before the addition of diphenylcarbazide.

(10) Phosphate, vanadate and selenate give remarkably higher results even in small amounts, because barium salts of these anions are also insoluble in water.

(11) Also arsenate, molybdate, selenite and thiosulfate give some interference even at 10 p. p. m.

However, the undesirable substances described above are not usually contained in common natural waters (rain water, river water etc.) in such concentrations as would give a serious error, therefore this rapid analytical method of sulfate may be applicable to the ordinary water analysis with satisfactory results.

Summary

The authors have devised a new method for the determination of small amounts of sulfate. Sulfate is precipitated as barium sulfate using barium chromate suspension in an acid solution (0.5 M acetic, 0.01 M hydrochloric acid), and the excess of barium is almost completely precipitated as barium chromate by adding ammonia water (containing calcium ion) and ethanol. Finally the amount of chromate which is exchanged with sulfate is estimated.

The proposed method may be applicable to the determination of 0.3—100 p. p. m. sulfate with satisfactory results.

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Stoichiometric Investigations of the Liquid State. V[*1]. *Vaporization Phenomenon of Associated Liquids*

By Kiyoshi TYUZYO[*2]

(Received May 9, 1957)

Reasonablesness of classifying associated liquids in two kinds was discussed in the previous paper[1]. In that paper, a method for determining the degree of association of associated liquid of the first kind from viscous flow was proposed and the association state was discussed in detail.

In this paper, a method for determining the degree of association from energy of vaporization and a method for calculating energy of hydrogen bond are proposed.

Energy of vaporization of *n*-paraffin

Relation between energy of vaporization at boiling point of *n*-paraffin ($E_{vap,b}$ in Kcal./mole) and molar volume at room temperature (V in cc./mole) is shown in Fig. 1. The following equation is established approximately in the range of V larger than 250.

$$E_{vap,b}=16\log V-28.4 \qquad (1)$$

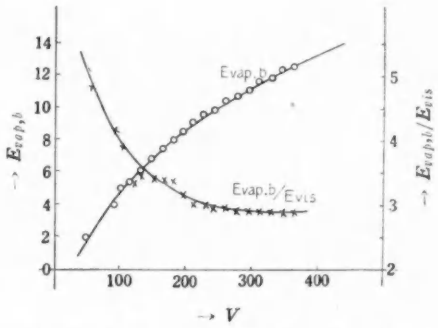

Fig. 1. $E_{vap,b}$ and $E_{vap,b}/E_{vis}$ vs. V of *n*-paraffins.

Relation between $E_{vap,b}/E_{vis}$ and V is shown in Fig. 1. This ratio decreases with increase in V and converges to about 2.8. $\log(E_{vap,b}/E_{vis}-2.80)$ vs. $\log V$ is approximately linear as is shown in Fig. 2. Eq. 2 is established from this.

$$E_{vap,b}/E_{vis}=2.80+(100/V)^2 \qquad (2)$$

This equation is applicable at least in the range of V from 60 to 370. According

*1) The fourth paper of this series, This Bulletin, **30**, 782 (1957).

*2) Present address, Research Laboratory, Dai-Nippon Celluloid Co., Sakai, Osaka

1) K. Tyuzyo, This Bulltein, **30**, 782 (1957).

to Eyring et al.[2], $E_{vap,b}/E_{vis}$ is about 3.5 for unassociated liquid, but this relation is not established rigorously as is shown in Fig. 1.

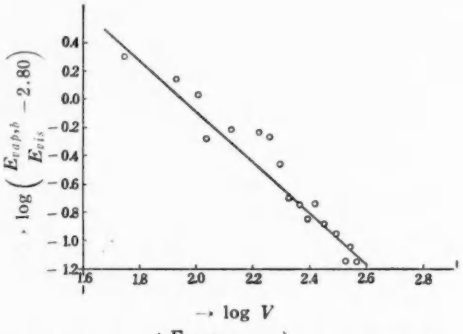

Fig. 2. $\log\left(\frac{E_{vap,b}}{E_{vis}}-2.80\right)$ vs. $\log V$ of n-paraffins.

Method for calculating degree of association of associated liquids of the first kind from energy of vaporization

Viscous flow of associated liquids of the first kind is closely analogous to that of n-paraffin as is shown in the previous paper. Therefore, if an associated body transfers from liquid to vapor phase without dissociation during vaporization, eq. 1 may be established between molar volume of associated body and energy of vaporization of this process. At the same time, eq. 2 may be established among this energy of vaporization, molar volume of associated body and energy of activation of viscous flow. Namely, when the molar volume of an associated body and the energy of vaporization of this process are respectively termed as V_a (cc/mole) and $E_{vap,a}$ (Kcal./mole), then

$$E_{vap,a}=f(V_a)$$
$$=16\log V_a-28.4 \text{ for } V_a>250 \quad (3)$$
$$E_{vap,a}=E_{vis}\cdot[2.80+(100/V_a)^2] \quad (4)$$
$$V_a=n\times V \quad (5)$$

where n is degree of association and V is normal molar volume.

V_a satisfying both eq. 3 and 4 can be obtained as is shown in Fig. 3. In the figure, curve-(a) is common for all associated liquids of the first kind and is expressed by eq. 3. Curve-(b) is characteristic for each associated liquid and is expressed by eq. 4. V_a is obtained from intersecting point of curve-(a) and (b). Then n is calculated from eq. 5.

Degree of association obtained from this method is termed as n_v and given in Table I. In the Table, n values from literature are also shown. It is clear from

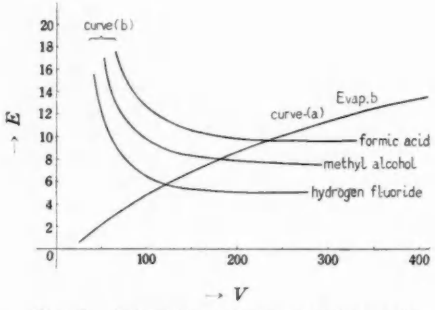

Fig. 3. Calculation of degree of association of formic acid, methanol and hydrogen fluoride.

TABLE I
DEGREE OF ASSOCIATION CALCULATED FROM EQ. 3 AND 4

Substance	V	V_a	n_v	n
saturated fatty acids				
formic acid	37.7	246	6.5	polymer
acetic acid	57.2	186	3.3	polymer
propionic acid	74.6	173	2.3	2
n-butylic acid	91.9	198	2.2	〃
n-valeric acid	108.7	238	2.2	〃
n-caproic acid	125.0	270	2.2	〃
n-heptylic acid	141.9	296	2.1	〃
n-caprylic acid	158.6	336	2.1	〃
n-pelargonic acid	174.5	394	2.2	〃
n-capric acid	196.2	423	2.2	〃
n-lauric acid	229.8	482	2.1	〃
n-myristic acid	270.4	523	1.9	〃
n-palmitic acid	304.6	564	1.9	〃
n-stearic acid	338.9	603	1.8	〃
saturated aliphatic alcohols				
methanol	40.5	182	4.5	5.0
ethanol	58.3	270	4.6	4.7
propanol	75.0	367	4.9	4.7
n-butanol	92.0	403	4.4	—
n-pentanol	107.7	500	4.6	—
n-hexanol	125.2	534	4.3	—
n-heptanol	140.7	606	4.3	—
n-octanol	156.7	673	4.3	—
n-cetanol	285	(955)	3.4	—
others				
hydrogen cyanide	38.5	118	3.1	3
hydrogen fluoride	20.4	122	6.0	6∼10
formamide	39.9	328	8.2	—

2) H. Eyring, *J. Chem. Phys.*, **4**, 283 (1936); R. H. Ewell and H. Eyring, ibid., **5**, 726 (1937).

the table that n_v is quite consistent with n. Literature of the n value is referred to in the previous paper. It may be understood from these facts that viscous flow of associated liquid of the first kind is closely analogous to that of n-paraffin.

A new method for calculating energy of hydrogen bonds

Vaporization process of associated liquid of the first kind is schematically shown in Fig. 4. In the figure,

State A: liquid system composed of 1 mole of associated body of n-mer just before vaporization

State B: hypothetical gas system composed of 1 mole of associated body of n-mer just after vaporization

State C: hypothetical liquid system composed of n/n' mole of associated body of n'-mer just before vaporization

State D: gas system composed of n/n' mole of associated body of n'-mer just after vaporization

E_1: dissociation energy of 1 mole of associated body of n-mer of liquid into n/n' mole of associated body of n'-mer of the liquid

E_2: Energy of vaporization of n/n' mole of associated body of n'-mer of the liquid

E_3: dissociation energy of 1 mole of associated body of n-mer of the gas into n/n' mole of associated body of n'-mer of the gas

E_4: energy of vaporization of 1 mole of associated body of n-mer of the liquid. This value is different from $E_{vap,a}$ in the preceding paragraph. Namely, E_4 is energy of vaporization at observed boiling point, compared with $E_{vap,a}$ which is energy of vaporization at the boiling point of n-paraffin corresponding to V_a.

E_5: energy required to produce n/n' mole of associated body of n'-mer of gas from 1 mole of associated body of n-mer of the liquid

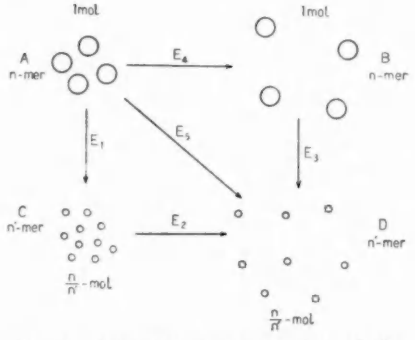

Fig. 4. Schematic representation of the vaporization process of the associated liquid of the first kind.

It is clear that the following equations are established.

$$E_1+E_3=E_4+E_2=E_5 \tag{6}$$

$$E_5=n\cdot\Delta E_{vap} \tag{7}$$

where ΔE_{vap} is observed energy of vaporization at an observed boiling point in Kcal./mole.

If the associated state is linear chain association,

$$E_3=N_A\cdot\left[(n-1)-\frac{n}{n'}(n'-1)\right]\cdot\varepsilon_{hb} \tag{8}$$

where ε_{hb} is energy of hydrogen bond per bond and N_A Avogadro's number. Namely,

$$E_3=\left(\frac{n}{n'}-1\right)\cdot E_{hb} \tag{9}$$

where E_{hb} is energy of hydrogen bond per mole in Kcal.

If the associated state is ring-like dimer association and $n=2$, $n'=1$,

$$E_3=2\cdot E_{hb} \tag{10}$$

E_4 can be calculated as follows. This is the energy of vaporization per mole of asociated body of n-mer at observed boiling point. It is widely known that the following equation is established between heat of vaporization and temperature for unassociated liquid[3].

$$\frac{\Delta H}{\Delta H_b}=\left(\frac{1-T/T_c}{1-T_b/T_c}\right)^{0.38} \tag{11}$$

where ΔH and ΔH_b are the heat of vaporization at T and T_b in °K respectively and T_c is critical temperature in °K. Therefore, energy of vaporization at T can be given by eq. 12.

$$\Delta E=(\Delta E_b+RT_b)\left(\frac{1-T/T_c}{1-T_b/T_c}\right)^{0.38}-RT \tag{12}$$

On the other hand, the following equations are established between T_b, T_c and V for n-paraffin.

$$T_c=664\log V-906 \tag{13}$$

$$T_b=620\log V-975 \tag{14}$$

It is clear from the previous paper and the preceding paragraph that an associated

3) K. M. Watson, *Ind. Eng. Chem.*, **35**, 398 (1943); K. Sato, *Kagaku Kogaku* (*Chemical Engineering*), **18**, 266 (1954) (in Japanese).

body of the first kind shows an analogous behavior to that of *n*-paraffin of equal molar volume. Therefore, critical temperature and boiling point of the associated body may be calculated by eq. 13 and 14. These are termed as $T_{c,a}$ and $T_{b,a}$. Then, E_i can be calculated by inserting $T_{c,a}$ and $T_{b,a}$ in eq. 12 and adopting observed boiling point as T. Namely,

$$E_i=(\Delta E_{b,a}+RT_{b,a})\left(\frac{1-T_b/T_{c,a}}{1-T_{b,a}/T_{c,a}}\right)^{0.38}-RT_b \tag{15}$$

where,

$$\Delta E_{b,a}=[2.80+(100/nV)^2]\cdot E_{vis} \tag{16}$$

$$T_{b,a}=620\log(nV)-975 \tag{17}$$

$$T_{c,a}=664\log(nV)-906 \tag{18}$$

TABLE II

CALCULATION OF ENERGY OF HYDROGEN BOND

Substance	E_{vis}	ΔE_{vap}	V	T_b	$T_{b,a}$	$T_{c,a}$	n	n'	E_{hb}	lit. for n'
saturated aliphatic alcohols										
methanol	2.61	7.73	40.5	338	440	631	5	1	7230	(4)
ethanol	3.57	8.75	58.3	351	532	732	〃	〃	7440	〃
propanol	4.39	9.08	75.0	370	615	804	〃	〃	6800	〃
n-butanol	4.64	9.60	92.0	391	637	864	〃	〃	7420	〃
n-pentanol	5.20	10.25	107.7	411	698	907	〃	〃	7420	〃
n-hexanol	5.40	10.75	125.2	429	715	951	〃	〃	8030	〃
n-heptanol	5.76	11.30	140.7	449	749	985	〃	〃	8270	〃
n-octanol	5.99	11.70	156.7	468	778	1017	〃	〃	8520	〃
saturated fatty acids										
formic acid	3.35	4.77	37.7	374	508	728	7	2	8430 3610	(5)
acetic acid	2.66	5.03	57.2	391	432	586	3	1.8	8580 360	(6)
propionic acid	2.47	6.48	74.6	414	412	586	2.3	(1.4)	11150 2600	
n-butylic acid	2.81	9.16	91.9	437	449	605	2	1.1	11300 5650	(7)
n-valeric acid	3.27	9.60	108.7	460	500	651	〃	(1)	8200 4100	
n-caproic acid	3.57	(9.96)	125.0	478	532	690	〃	(1)	7850 3930	
n-heptylic acid	3.84	(10.40)	141.9	496	558	725	〃	1	7860 3930	(7)
n-caprylic acid	4.18	(10.85)	158.6	512	594	756	〃	(1)	7120 3560	
n-pelargonic acid	4.58	(11.28)	174.5	527	633	784	〃	(1)	5990 3000	
n-capric acid	4.76	(11.86)	196.2	541	653	816	〃	(1)	6530 3270	
n-lauric acid	5.10	12.58	229.8	574	688	864	〃	(1)	7030 3520	
n-myristic acid	5.34	13.50	270.4	601	709	911	〃	(1)	8700 4350	
n-palmitic acid	5.54	(14.01)	304.6	628	731	944	〃	(1)	9380 4690	
n-stearic acid	5.74	14.56	338.9	658	747	974	〃	(1)	10480 5240	
others										
hydrogen cyanide	1.64	6.05	38.5	299	306	462	3	1.1	7070	(8)
hydrogen fluoride	1.69	6.46	20.4	293	315	481	6	1.8	14000	(9)

4) L. Grunberg and A. H. Nissan, *Trans. Faraday Soc.*, **45**, 125 (1949).
5) R. Wolff, *Angew. Chem.*, **67**, 89 (1955).
6) M. Toda, Structure of Liquid, p. 51 (1949) (in Japanese); Ref, (6).
7) R. E. Lundin, F. E. Harres and L. K. Nash, *J. Am. Chem. Soc.*, **74**, 743 (1952).
8) W. F. Giauque and R. O. Ruehervin, ibid., **61**. 2626 (1939).
9) J. Simon and J. H. Hildebrand, ibid., **46**, 2183 (1924); R. W. Long, J. H. Hildebrand and W.E. Morrell, ibid., **65**, 182 (1943).
10) S. Seki, *Chemistry and Chemical Industry*, **6**, 182 (1953) (in Japanese); L. N. Fergusson, Electron "Structures of Organic Molecules," p. 57 (1952).

TABLE III
ENERGY OF HYDROGEN BOND

Substance	State	Method	Energy (Kcal./mole)
Hydrogen fluoride	gas	vapor density	6∼10
Fatty acid	gas	vapor density	7∼ 9
Alcohol	liquid	heat of vapor.	6∼ 7
	gas	heat conduct.	7∼ 8
Hydrogen cyanide	gas	vapor density	3∼ 4
Water	s, l, g	heat of vapor.	4∼ 5
		second virial	
Ammonia	gas	second virial	3∼ 4
	liquid	dielectric constant	6
	solid	heat of sublim.	1.3
Acetamide	solid	heat of sublim.	3.5
Aniline		partition coeff. (CCl_4—H_2O)	1.93

Following equations may be deduced from equation 6, 7, 9, 10 and 15.

(i) linear chain association

$$(\Delta E_{b,a}+RT_{b,a})\left(\frac{1-T_b/T_{c,a}}{1-T_{b,a}/T_{c,a}}\right)^{0.38}-RT_b+\left(\frac{n}{n'}-1\right)\cdot E_{hb}=n\Delta E_{vap} \quad (19)$$

(ii) ring-like dimer association

$$(\Delta E_{b,a}+RT_{b,a})\left(\frac{1-T_b/T_{c,a}}{1-T_{b,a}/T_{c,a}}\right)^{0.38}-RT_b+2E_{hb}=2\Delta E_{vap} \quad (20)$$

Using eq. 19 and 20, one of the values, n, n' and E_{hb} can be calculated from the remaining two, when V, E_{vis}, T_b and ΔE_{vap} are known.

In Table II are shown T_b, $T_{b,a}$ and $T_{c,a}$ from eq. 17 and 18, n, n' and E_{hb} from eq. 19 and 20 and literature for n'. Moreover, energy of hydrogen bond from literature is shown in Table III.

The following is clear from the tables.

1) Calculated values of energy of hydrogen bond of alcohols are quite consistent with those from literatures and are independent of the length of carbon chain.

2) Calculated values of energy of hydrogen bond of formic- and acetic acid are quite consistent with those from the literature of the subject if the associated state is linear chain association, but are quite inconsistent if the associated state is ring-like dimer association. This fact suggests linear chain association of these compounds in the liquid state.

3) In propionic- and n-butylic acid, E_{hb} values based on linear chain association are too great and those based on ring-like dimer association are too small. This fact suggests mixture of linear chain and ring-like association in the liquid state. These results are quite consistent with those in the previous paper.

4) In higher fatty acids, calculated values of E_{hb} are fairly consistent with those from literature, which assumes linear chain association. However, a decisive answer cannot be given because of the lack of accurate data of degree of association in the gaseous state, n'. E_{hb} based upon ring-like dimer association may be more consistent with the observed value even if n' is slightly greater than unity.

5) Calculated value of E_{hb} of hydrogen cyanide is about two times as great as that from literature. However, accurate data of E_{hb} of this compound are very rare. Moreover, E_{hb} of water is greater than that of hydrogen cyanide from literature, though water belongs to the associated liquid of the second kind and its hydrogen bond is easily destroyed with increase in temperature according to the previous paper. Considering these facts, the E_{hb} value of hydrogen cyanide from literature seems to be too small. A detailed investigation may be required.

6) Calculated value of E_{hb} of hydrogen fluoride is some what greater than that from literature, but the cause is not clear at present. However, the value from literature is not quite accurate.

The starting equation is $E_4+E_1=E_3$ in the above treatment, but it is of course possible to start from the equation, $E_1+E_2=E_3$. However, E_1 does not consist of the energy required to destroy hydrogen bond, because this process is a dissociation reaction in the liquid state. Moreover, eq. 2 which is established in the case of n-paraffin is not always applicable to E_2, because E_2 is the energy of vaporization

of a polar liquid. Therefore, the method based on E_1 and E_2 is more difficult than that based on E_1 and E_3. At any rate, it is very remarkable that energy of hydrogen bond can be calculated as shown above, on the assumption that the associated body of the first kind is analogous to *n*-paraffin.

Summary

1) Energy of vaporization of *n*-paraffin is discussed. It is shown that relation between ΔE_{vap} at the boiling point and E_{vis} is different from that of Eyring et al..

2) A new method for calculating the degree of association of the first kind from energy of vaporization is proposed. The degree of association calculated by this method is quite consistent with the result of the previous paper and the value from literature.

3) A new method for calculating the energy of hydrogen bond is proposed. Energy of hydrogen bond obtained by this method is fairly consistent with the value from literature.

4) Considering all results, it can be concluded that the associated body of the first kind shows analogous behavior to *n*-paraffin.

The author wishes to express his sincere thanks to Professor N. Sata of Osaka University for his kind instruction.

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Dye-sensitized Photopolymerization of Acrylonitrile-Vinyl Acetate Copolymers

By Masakazu TANIYAMA and Gerald OSTER

(Received June 3, 1957)

It has been discovered that vinyl polymerization may be initiated by irradiating with visible light a solution containing certain dyes and a mild reducing agent[1]. Oxygen is necessary for the process. The dye is photoreduced to its leuco form and, on reacting with ambient oxygen free, radicals are produced which initiate the polymerization.

In all the studies carried out with such an initiator, very high molecular weight polymer is obtained. For example, the use of riboflavin (a naturally occurring dye-reducing agent combination) as the photosensitizer results in the polymerization of acrylamide to give a polymer having a molecular weight exceeding ten millions[2]. Copolymers of acrylonitrile and allyl alcohol have been produced by dye-sensitized photopolymerization having molecular weights far in excess of those produced by conventional catalysts[3].

It is the purpose of the present work to study the copolymerization of acrylonitrile and vinyl acetate by dye sensitization under conditions which resemble those carried out using conventional catalysts (e. g., organic peroxides)[4] in order to compare reactivity ratios obtained by the two different methods. A practical consequence of the present work may be to suggest a convenient method of producing these polymers which may be controlled simply by suitably adjusting the light intensity.

Experimental

Materials. — Acrylonitrile (American Cyanamid) and vinyl acetate (Eastman Kodak) having boiling points of 77° and 72°C, respectively, were distilled under helium to remove inhibitor. The dye used was acriflavine (National Aniline) and the reducing agent was allyl thiourea (Eastman Kodak). Dimethylformamide (Matheson) was used as the solvent.

1) G. Oster, *Nature*, **173**, 300 (1954).
2) G. K. Oster, G. Oster and G. Prati, *J. Am. Chem. Soc.*, **79**, 595 (1957).
3) G. Oster and Y. Mizutani, *J. Polymer Sci.*, **22**, 173 (1956).
4) C. G. Swain and P. D. Bartlett, *J. Am. Chem. Soc.*, **68**, 2381 (1946); G. M. Burnett and H. W. Melville, *Proc. Roy. Soc.* (London), **A189**, 456 (1947); G. Dixon-Lewis, ibid., **A198**, 510 (1949); M. S. Matheson, E. E. Auer, E. Bevilacqua and E. J. Hart, *J. Am. Chem. Soc.*, **71** 2610 (1949); H. Kwart, H. S. Broadbent and P. D. Bartlett, ibid., **72**, 1060 (1950); F. R. Mayo, F. M. Lewis, C. Walling, and W. F. Hulse, ibid., **70**, 1523 (1948).

TABLE I

SOME PROPERTIES OF THE COMONOMER AND COPOLYMER SYSTEMS

Exp. No.	1	2	3	4	5	6	7	8	9	10
Acrylonitrile molar ratio in initial mixture	1.000	0.936	0.867	0.792	0.708	0.618	0.519	0.410	0.289	0.153
Acrylonitrile molar ratio in copolymer	1.000	0.959	0.978	0.954	0.912	0.850	0.816	0.767	0.704	0.618
Relative rate of conversion	8.14	9.78	12.25	14.68	14.78	12.07	5.90	3.48	2.61	1.61
Intrinsic viscosity of copolymer (dl./gm.)	1.239	0.907	0.810	0.690	0.752	0.733	0.879	0.672	0.474	0.364
4.46 μ/5.75 μ infra red absorbance	—	—	1.352	0.865	0.739	0.662	0.602	0.530	0.495	0.467

Procedure and Results.—A total of 10 g. of mixtures in various proportions of acrylonitrile and vinyl acetate was added to 15 ml. of dimethylformamide. To the solution were added the dye and the reducing agent to give a final concentration of 0.00625% and 0.04%, respectively. The final solution was placed in a 50 ml. test tube at a distance 45 cm. from a 500 watt projection lamp. At various times during the irradiation samples were withdrawn and added to a 50—50 mixture of methanol and water. The precipitate was washed with the methanol-water mixture and dried in a vacuum oven at 55°C. The amount of monomer converted as a function of time of irradiation for the various initial mixtures are illustrated in Fig. 1 and the relative rates are given in Table I.

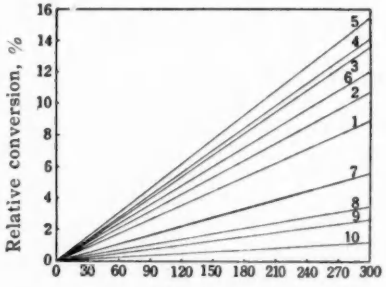

Fig. 1. Conversion as a function of time of irradiation for various initial monomer mixtures.

Curve No.	1	2	3	4	5
acrylonitrile molar ratio	1.000	0.936	0.867	0.792	0.708
Curve No.	6	7	8	9	10
acrylonitrile molar ratio	0.618	0.519	0.410	0.289	0.153

When the molar ratio of acrylonitrile in the original mixture exceeds 0.792, the system becomes turbid during irradiation. In such cases the suspensions were agitated prior to the withdrawal of samples.

For other studies (see below) the samples were purified by first dissolution in dimethylformamide, precipitation and repeated washing with the alcohol-water mixture.

Nitrogen analyses were carried out by the Kjeldahl method and the results obtained were corrected according to the procedure of Nozaki[5]. The nitrile content of the polymer, as determined from the nitrogen analyses, are given in Fig. 2 as a function of acrylonitrile concentration in the starting mixture.

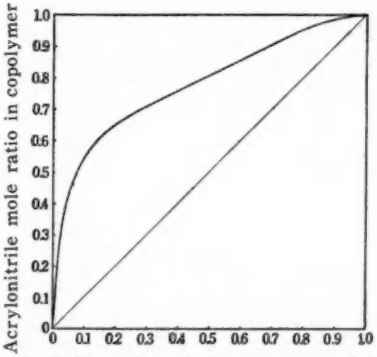

Fig. 2. Copolymerization curve. The straight line represents that expected for an "ideal" copolymerization system.

All the copolymers studied were soluble in dimethylformamide. The copolymers, obtained when the initial molar ratio was 0.519 or greater in acrylonitrile, exhibited only slight swelling in acetone. On the other hand, for a ratio of 0.410, the copolymer was swollen by acetone and for a ratio of 0.289, the copolymer was completely soluble in acetone. The viscosities of solutions of the copolymers in dimethylformamide at 30°C. were determined at 30°C. were determined in a Ubbelohde viscometer with a flow time of 300 sec. The intrinsic viscosities, calculated in the usual manner, are given in Table I.

Samples of the polymers were prepared for infrared analysis by mixing 5 mg. of the polymers with 500 mg. of dried potassium bromide and made into a disc tablet by means of a hydraulic press. In Fig. 3 are illustrated the infrared spectra as obtained on a Perkin-Elmer Model 21 Infra Red spectrometer.

5) K. Nozaki, *J. Polymer Sci.*, **1**, 455 (1946).

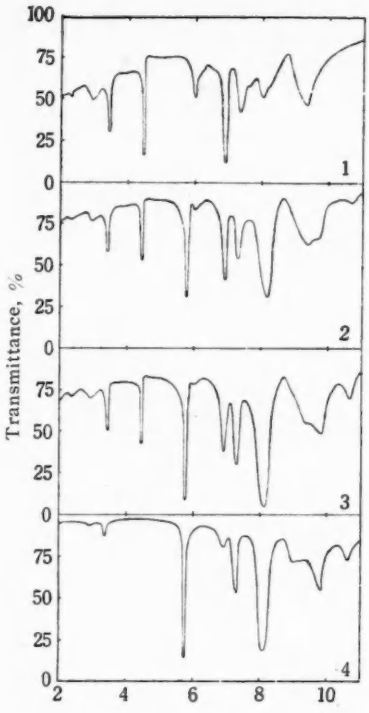

Fig. 3. Infra red spectra of the polymers having the molar ratios of acrylonitrile in the polymers of (1) 1.000, (2) 0.954, (3) 0.767, and (4) 0, respectively.

Discussion

It is instructive to represent the data of Fig. 1 in terms of conversion as a function of acrylonitrile content in the initial mixture (Fig. 4). A maximum rate is obtained when the molar ratio of acrylonitrile is about 0.70. If the intrinsic viscosity-molecular weight relation is the same for all the polymers, (which it probably is not) then the molecular weight is the greatest for the pure acrylonitrile system. In any case, the molecular weights probably do not exceed 20,000. On the other hand, dye sensitized photopolymerization of acrylonitrile in saturated zinc chloride solutions yields polymer with molecular weights exceeding one million[6].

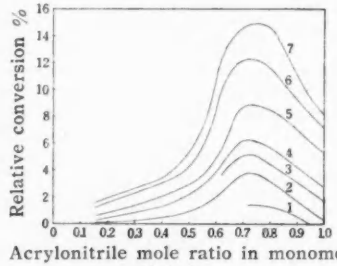

Fig. 4. Conversion of monomer as a function of acrylonitrile in original mixture.

Curve No.	1	2	3	4	5	6	7
Time of irradiation (min.)	30	60	90	120	180	240	300

The infra red spectra of the polymers shown in Fig. 3 are consistent with the nitrogen analysis. With decreasing nitrile content in the copolymer, the absorption band characteristic of that group at 4.46 μ is suppressed as the absorption band characteristic of the carbonyl group of acetate at 5.75 μ increases. Hence the ratio of these two absorption peaks decreases as the nitrogen content decreases (see Table I). Other significant changes in the infra red spectra take place in the 9—10 μ region.

From the data in Fig. 2 we calculate the reactivity ratios r_1 and r_2 (for acrylonitrile and vinyl acetate, respectively) using the well known expression[7],

$$F_1 = \frac{r_1 f_1^2 + f_1 f_2}{r_1 f_1^2 + 2 f_1 f_2 + r_2 f_2^2}$$

where F_1 is the acrylonitrile molar ratio in the copolymer, and f_1 and f_2 are the molar ratios of acrylonitrile and vinyl acetate, respectively, in the original mixture. By curve fitting methods, we obtain the values $r_1=3.88$ and $r_2=0.009$. These results differ somewhat from those of Mayo et al.[4] who obtain values of $r_1=4.05$ and $r_2=0.016$ for bulk polymerization of acrylonitrile-vinyl acetate mixtures using peroxide catalysts.

The copolymer having a molar ratio of acrylonitrile of 0.704 (initial mixture ratio of 0.289) is soluble in acetone and hence could be dry spun from this inexpensive solvent. It is interesting that although this polymer is rich in nitrile groups it is, nevertheless, soluble in acetone. Apparently the polymer has random alternation of nitrile and acetate groups since the product $r_1 r_2$ is small.

Summary

Copolymers of acrylonitrile and vinyl acetate have been produced using a dye-

6) G. Oster and H. Lieberman, to be published.
7) I. Skeist, *J. Am. Chem. Soc.*, **68**, 1781 (1946).

reducing agent combination as the photosensitizer. Visible light was employed as the radiation source. Nitrogen analyses and infra red analyses were performed on the polymers obtained. The copolymerization ratios were 3.88 and 0.009 for acrylonitrile and vinyl acetate, respectively. Below an initial monomer molar ratio of acrylonitrile of 0.3, the resultant copolymer dissolves in acetone.

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Estrogenic Biphenyls. III. 2-Alkyl-4-methoxybiphenyl-4'-carboxylic Acids

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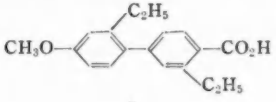
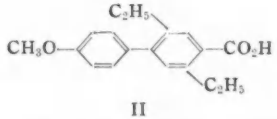
(Received June 3, 1957)

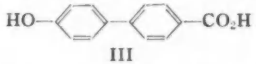
In the previous papers[1,2] of this series, it was reported that 2, 3'-diethyl-4-methoxybiphenyl-4'-carboxylic acid(I) and 2', 5'-diethyl-4-methoxybiphenyl-4'-carboxylic acid(II) were fully estrogenic at the doses of 100 γ when administered to ovariectomized mice. Enhancement of the estrogenic activity of compounds I and II, compared with that of 4-hydroxybiphenyl-4'-carboxylic acid(III), by the presence of the two ethyl groups was attributed to the steric effect causing the two benzene rings to rotate out of the otherwise coplanar structure and the increasing thickness of the molecules, which was shown by the hypsochromic effect in the ultraviolet absorption spectra.



I



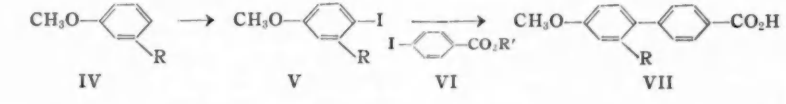
II



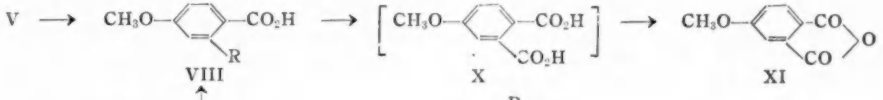
III

It is unknown, however, whether the introduction of ethyl groups into 4-hydroxy- or 4-methoxy-biphenyl-4'-carboxylic acid at positions 2 and 3' or 2' and 5' is the optimum substitution for developement of the estrogenic activity. Therefore, the authors hoped to find out first the most suitable alkyl group substituted at position 2 only of 4-methoxybiphenyl-4'-carboxylic acid.

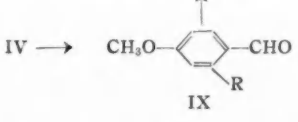
2-Alkyl-4-methoxybiphenyl-4'-carboxylic acids(VII) were synthesized in the following way. 3-Alkylanisoles(IV) were iodinated with iodine in the presence of yellow



IV V VI VII



VIII X XI



IX

R
a H
b CH_3
c CH_3CH_2
d $CH_3CH_2CH_2$

* Present address : Department of Chemistry, Faculty of Science, The University of Tokyo, Hongo, Tokyo.

1) M. Ōki and T. Sato, This Bulletin, **30**, 508 (1957).
2) M. Ōki and T. Sato, ibid., 702.

mercuric oxide[3]. The structures of 3-alkyl-4-iodoanisoles(V), compounds Va[3] and Vc[1] being known, were proved as follows:

The reaction of Grignard compound from 4-iodo-3-methylanisole (Vb) with solid carbon dioxide produced a carboxylic acid. It was identified with 4-methoxy-2-methylbenzoic acid (VIIIb) which was prepared by permanganate oxidation of 4-methoxy-2-methylbenzaldehyde (IXb), obtained from 3-methylanisole(IVb), zinc cyanide, hydrogen chloride and aluminium chloride[4]. The acid (VIIId) obtained from 4-iodo-3-propylanisole (Vd) in the same way was oxidized with potassium permanganate. The product was sublimed in vacuo to produce an anhydride, which was identical with 4-methoxyphthalic anhydride (XI) prepared from 4-methoxy-2-methylbenzoic acid (VIIIb). 3-Alkyl-4-iodoanisoles (V) and 4-iodobenzoic esters (VI) were submitted to the Ullmann reaction. Any unchanged materials were removed in vacuo when necessary, and hydrolysis of the condensation products gave the desired biphenyls (VII).

TABLE I

ULTRAVIOLET ABSORPTION MAXIMA AND ESTROGENIC ACTIVITY OF 2-ALKYL-4-METHOXYBIPHENYL-4'-CARBOXYLIC ACID (VII)

Compound	$\lambda_{max.}$ (mμ)	log $\varepsilon_{max.}$	Minimum active dose in mice (γ)
VIIa[5]	289	4.36	1000*
VIIb	224 280	4.27 4.17	200
VIIc	222 278	4.28 4.10	500
VIId	224 278	4.28 4.13	250

* Active in 50% animals.

The ultraviolet absorption data and the estroganic activity of compounds VIIa, VIIb, VIIc, and VIId are given in Table I. 4-Methoxybiphenyl-4'-carboxylic acid (VIIa) showed a strong absorption at 289 mμ, while its homologs (VIIb, c, d) possessed absorption at about 278 mμ. It can be deduced from the spectral study that the introduction of an alkyl group into position 2 modifies the coplanar conformation of the biphenyl skeleton, because the observed hypsochromic shift and decrease in K-band intensity in the present series (VII) can be attributed to the non-planar conformation of the system, if the wave length of the maximum absorption depends on the extent of conjugation. Further, it may be inferred that the deterioration in conjugation in the series (VII) is similar regardless of the length of the alkyl chains, and then, it may be assumed that the first methylene group only interferes with the benzene rings. The present observation is in good agreement with others[6]. Namely, it is known that by a single substitution at position 2 of biphenyl a little change occurs in the ultraviolet absorption and the K-band appears, though the maximum shifts to a shorter wave length and the intensity diminishes, and that 2-methylbiphenyl absorbs at 237 mμ and 2-ethylbiphenyl at 233 mμ while biphenyl itself does at 249 mμ.

The minimum active doses of the members of the series VII to produce full estrus were determined by the vaginal smear test with ovari-ectomized mice. The substances in oil solution were injected subcutaneously. 4-Hydroxybiphenyl-4'-carboxylic acid (III) produced estrus in 60% of the test animals at the dose of 500 γ but the assay could not be carried out at 1 mg. dose because of its small solubility in oil[1]. On the other hand, compound VIIa was found to be active to 40% and 50% of the animals at the doses of 500 γ and 1 mg., respectively. Natural estrogens, hexestrol, and stilbestrol, lose potency markedly when they are converted into methyl ethers[7], while there is no remarkable difference in estrogenic activity between a free phenol and its methyl ether of the type of doisynolic acid[8] or allenolic acid[9]. 4-Hydroxybiphenyl-4'-carboxylic acid, and presumably its 2-alkylhomologs, fall in the latter category both in the two functional groups and in the effect of the methylation of the hydroxyl group. It is striking, however, that the kind of the alkyl group gives almost no difference in the estrogenic activity in contrast to the case of dialkylstilbestrol dimethyl ethers[10]. This may be understood on the basis of the molecular thickness being similar irrespective of the kind

3) M. P. Brenans, *Bull. Soc. chim. France*, [3] **25**, 819 (1901).
4) R. Adams and E. Montgomery, *J. Am. Chem. Soc.*, **46**, 1518 (1924).
5) Prepared according to the method given by L. F. Fieser and C. K. Bradsher, ibid., **58**, 1738 (1936).
6) E. A. Braude, F. Sondheimer and W. F. Forbes, *Nature*, **173**, 117 (1954) : R. A. Friedel, M. Orchin and L. Reggel, *J. Am. Chem. Soc.*, **70**, 199 (1948) : C. D. Freedman, ibid., **77**, 6223 (1955).
7) U. V. Solmssen, *Chem. Rev.*, **37** 481 (1945).
8) K. Miescher, ibid., **43**, 367 (1948),
9) See A. Horeau and Jacque, *Bull. Soc. chim. France*, **1945**, 1001 and the following papers.
10) M. Ōki and Y. Urushibara, This Bulletin, **25**, 109 (1952).

of the alkyl group as judged from the ultraviolet absorption, provided that the molecular thickness is mainly given by the rotated benzene rings.

Experimental[11]

3-Propylaniline. A mixture of 130 g. (0.87 mole) of 3-aminopropiophenone, prepared from 3-nitropropiophenone according to Kenford and Simpson[12], 120 g. (1.8 moles) of 72% hydrazine hydrate, 280 g. of potassium hydroxide and 700 ml. of diethylene glycol, was heated under reflux for one hour. The reflux condenser was replaced by a downward condenser. Distillation was continued until the pot temperature rose to 190° and the mixture was maintained at this temperature for three hours. Then the mixture was steam-distilled. The combined distillate was extracted with ether and the extract was dried over potassium carbonate. Distillation gave a colorless oil boiling at 102—107°/8 mm. Yield 113 g. or 96% of the theoretical. The reported boiling point is 118—119°/12—13 mm.[13]

The picrate, prepared in the usual way, melted at 155—156°. The reported melting point is 155°[13].

Anal. Found: N, 15.83. Calcd. for $C_{15}H_{16}N_4O_8$: N, 16.09%.

3-Alkyl-4-iodoanisole (V). The general procedure is described by an example of the preparation of 4-iodo-2-methylanisole. To a solution of 28 g. (0.23 mole) of 3-methylanisole (IVb)[14] in 46 ml. of ethanol were alternatively added 58 g. (0.23 mole) of iodine and 60 g. (0.28 mole) of yellow mercuric oxide in small portions during ten to fifteen minutes, while the reaction mixture was kept at the temperature below 25° and the flask was well swirled. Then the mixture was heated under reflux for ten minutes, cooled, and filtered with suction. The filtrate was diluted with water and the oil was extracted with ether. The ethereal extract was washed with aqueous potassium iodide, dried over calcium chloride, and fractionally distilled. 4-Iodo-2-methylanisole (Vb) boiled at 123—125°/7 mm. and solidified on cooling. The analytical sample was obtained by recrystallization from ethanol. Colorless needles, m. p. 43—45°. Yield 31 g. or 54% of the theoretical.

Anal. Found: I, 51.26. Calcd. for C_8H_9OI: I, 51.18%.

3-Ethyl-4-iodoanisole (Vc) was prepared as described elsewhere[1].

4-Iodo-2-propylanisole (Vd) was obtained from 2-propylanisole (IVd)[15]. Colorless oil, b. p. 120—121°/4 mm. Yield 62%.

Anal. Found: C, 43.77; 4.85. Calcd. for $C_{10}H_{13}IO$: C, 43.50; H, 4.75%.

11) All melting and boiling points are uncorrected.
12) J. R. Kenford and J. E. Simpson, *J. Chem. Soc.*, **1948**, 354.
13) R. Long and K. Schofield, ibid., **1953**, 2066.
14) c. f. G. S. Hiers and F. D. Hager, *Org. Synth.*, Coll. Vol. **1**, 58 (1940).
15) 3-Propylanisole was prepared from the corresponding phenol which was obtained by the diazo-reaction of 3-propylaniline.

4-Methoxy-2-methylbenzoic Acid (VIIIb). *A. From 4-Methoxy-2-methylbenzaldehyde (IXb).* The benzaldehyde, prepared according to Adams and Montgomery[4], was oxidized with aqueous potassium permanganate to give colorless plates melting at 173°. The reported melting point is 176°[16].

Anal. Found: C, 65.23; H, 6.11. Calcd. for $C_9H_{10}O_3$: C, 65.05; H, 6.07%.

B. From 4-Iodo-2-methylanisole (Vb). 4-Methoxy-2-methylphenylmagnesium iodide was prepared in the usual way from 5 g. of compound Vb, 0.6 g. (0.029 atom) of magnesium turnings and 50 ml. of ether. Solid carbon dioxide was added to this solution and the product was decomposed with dilute hydrochloric acid. The acid in the ethereal layer was taken up in aqueous sodium bicarbonate and the aqueous solution was acidified with hydrochloric acid. The acid was recrystallized from aqueous ethanol. Colorless plates, m. p. 176—177°. Yield 2 g. or 60% of the theoretical. The melting point did not depress when this compound was mixed with 4-methoxy-2-methylbenzoic acid.

4-Methoxy-2-propylbenzoic Acid (VIIId). 4-Methoxy-2-propylphenylmagnesium iodide, prepared from 7 g. (0.025 mole) of compound Vd and 0.8 g. (0.033 atom) of magnesium in 50 ml. of ether was treated with solid carbon dioxide and the reaction mixture was worked up as in the preparation of compound VIIIb. Colorless plates, m. p. 106—107°, were obtained on recrystallization of the product from aqueous ethanol. Yield 2 g. or 41% of the theoretical.

Anal. Found: C, 68.03; H, 7.46. Calcd. for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27%.

4-Methoxyphthalic anhydride (XI). *A. From 4-Methoxy-2-methylbenzoic Acid (VIIIb).* The acid VIIIb was oxidized according to Shall[16] and the dicarboxylic acid (X) was sublimed in vacuo. Colorless needles, m. p. 88—90°. The reported melting point is 93°.

B. From 2-Ethyl-4-methoxybenzoic Acid (VIIIc). A solution of 500 mg. of compound VIIIc, 3 g. of potassium permanganate and 1 ml. of 10% sodium hydroxide in 60 ml. of water, was heated under reflux for three hours. Sulfur dioxide was bubbled into the mixture and the resulting solution was extracted with ether for five hours. After concentration of the ethereal extract, the residue was heated in vacuo and the crystalline material was collected. The compound melted at 90—90.5° and showed no depression of the melting point on admixture with the authentic specimen of 4-methoxyphthalic anhydride obtained from 4-methoxy-2-methylbenzoic acid as described above.

C. *From 4-Methoxy-2-propylbenzoic Acid (VIIId).* The acid VIIId was treated in the same way as described above. The ahnydride obtained melted at 85—86° and showed no depression of the melting point when mixed with the authentic 4-methoxyphthalic anhydride.

2-Alkyl-4-methoxybiphenyl-4'-carboxylic Acid (VII). 4-Iodobenzoic esters (VI) were

16) C. Shall, *Ber.*, **12**, 816 (1879).

obtained from 4-iodobenzoic acid in the usual way. Methyl ester, m. p. 113—114°[17] and ethyl ester, b. p. 154°/16 mm.[18] were prepared.

For the general procedure of the preparation, 4-methoxy-2-methylbiphenyl-4'-carboxylic acid (VIIb) is described as follows. To a well stirred mixture of 10 g. (0.040 mole) of 4-iodo-3-methylanisole (Vb) and 5 g. (0.019 mole) of methyl 4-iodobenzoate, 15 g. of activated copper bronze[19] was added in twenty-five minutes, the temperature being kept at 240—250°. The reaction mixture was then heated at 280—290° for twenty-five minutes and extracted with acetone after cooling. The solvent was removed by distillation and the residue was hydrolyzed by refluxing with a mixture of 50 ml. of 10% aqueous sodium hydroxide and 100 ml. of ethanol for two hours. Ethanol was evaporated and the residue was diluted with 500 ml. of water. The oily insoluble material was removed by filtration with the aid of active charcoal and the filtrate was acidified with hydrochloric acid. The precipitate was collected and boiled with acetic acid, and any insoluble material was removed by filtration. The extract was concentrated and the residue was again treated with boiling benzene. The filtrate gave brown crystals, which yielded colorless plates, m. p. 211—212°, on recrystallization from benzene. Yield 0.4 g. or 9% of the theoretical.

Anal. Found: C, 74.05; H, 5.76. Calcd. for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83%.

2-Ethyl-4-methoxybiphenyl-4'-carboxylic Acid (VIIc). It was obtained from compounds Vc and VI ($R'=C_2H_5$). It was necessary to remove any unchanged ester in vacuo before hydrolysis for easier purification of the desired material. The acid was finally recrystallized from aqueous ethanol. M. p. 173.5—174.5°. Yield about 7% of the theoretical.

Anal. Found: C, 74.56; H, 6.21. Calcd. for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29%.

4-Methoxy-2-propylbiphenyl-4'-carboxylic Acid (VIId). It was prepared in the same way as described for compound VIIc, starting from 4-iodo-2-propylanisole (Vd) and ethyl 4-iodobenzoate. M. p. 175.5—176°. Yield about 8% of the theoretical.

Anal. Found: C, 75.52; H, 7.03. Calcd. for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71%

Ultraviolet Absorption Spectra. They were measured by using Hitachi Photo-electric Spectrophotometer Model EPU-2. Substances were dissolved in 95% ethanol.

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17) P. P. T. Sah and Chang-lin Hsü, *Rec. Trav. Chim.*, **59**, 349 (1940).
18) K. Kindler, *Ann.*, **450**, 1 (1926).
19) E. C. Kleiderer and R. Adams, *J. Am. Chem. Soc.*, **55**, 4219 (1933).

*Über den Zucker des Narcissins**

Von Munio KOTAKE und Hisao ARAKAWA

(Eingegangen am 17 März 1957)

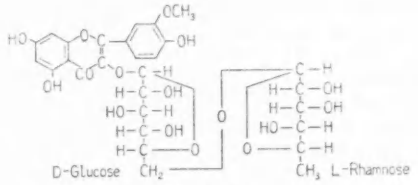
Es wurde das Narcissin von Kubota und Hase[1] als Isorhamnetin-3-rutinosid vermutet und in dem vorgehenden Berichte hat einer von uns[2] mit Hilfe der Papierchromatographie zeigen können, dass der Zucker des Narcissins mit der Biose des Rutins identisch ist, aber eine Gewinnung seines kristallinischen Derivates als Biose ist noch nicht gelungen. Es ist das Ziel dieser Untersuchung, ein kristallinisches Derivat der Biose des Narcissins zu isolieren und mit demselben der Rutinose zu vergleichen.

Zur Vorprobe der Spaltung unterwarfen wir das Rutin einer Ozonisierung nach Zemplén et al[3], da seine Ozonspaltung in der Literatur noch nicht beschrieben worden ist, und so wurden Oxalsäure und Zucker gewonnen; letzterer wurde nach Acetylierung mit dem synthetischen Präparat als Rutinose-heptaacetat identifiziert.

* III. Mitteilung über die Flavonolglykoside aus den Pollen von *Lilium auratum* RINDLE.
1) T. Kubota und T. Hase, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **77**, 1059 (1956).
2) H. Arakawa, ibid., **77**, 1314 (1956).
3) G. Zemplén et al, *Ber.*, **75**, 489 (1942).

Auf die gleiche Weise ozonisierten wir das Narcissin aus den Pollen[4] und bei der Papierchromatographie ergab sich nur ein Fleck, dessen R_f Wert mit demselben des Entacetylierungsproduktes vom Rutinose-heptaacetat übereinstimmte; nach Acetylierung konnte der Zucker des Narcissins als Rutinose charakterisiert werden.

Nun ist es nachgewiesen worden, dass Narcissin Isorhamnetin-3-rutinosid ist.



Narcissin

Beschreibung der Versuche

Rutinose-Heptaacetat (Hepta-*O*-acetyl-6-β-L-Rhamnopyranosyl-β-D-Glukopyranose) und seine Entacetylierung: Die nach Zemplén et al.[5] dargestellte Rutinose-heptaacetat schmolz bei 169,7°C; $[\alpha]_D^{21} = -28,8°$ ($c = 3,27$ in Chloroform). (Lit. Schmp. 168—169°C; $[\alpha]_D^{20} = -28,84°$[6]) Das IR-Spektrum zeigte keine α-Bindung, wohl aber die β-Glykosidbindung bei 11,19 μ, den β-Anomer des Glukose-Acetates** bei 9,64 μ (in Nujol).

Acetat (200 mg) wurde in 13 cc., 0,02 N Natriummethylat gelöst und 6 Std. bei Zimmertemperatur stehen gelassen; danach wurden Amberite IR 4B und IR 120 und dann Wasser unter Rühren durch Einleitung von Kohlendioxyd versetzt, abfiltriert und im Vak.-Exsiccator getrocknet. Das erhaltene Produkt wurde in 0,5 ccm Wasser gelöst und mit 10 ccm absol. Alkohol und 10 ccm absol. Äther versetzt; dabei fiel ein farbloser Niederschlag aus, der mit absol. Äther und absol. Chloroform gewaschen und getrocknet wurde. Er schmeckte etwas süss und ergab bei Papierchromatographie den einzigen Fleck der Rutinose[2], zeigte sich aber sehr hygroskopisch und noch nicht analysierein.

Ozonspaltung des Rutins: (Rutin[7] 1,5 g) wurde in 80 ccm 90 proz. Essigsäure 15 Std. ozonisiert. Die Lösung wurde dann im Vak. eingeengt. Der erhaltene Rückstand wurde mit Alkohol verdünnt und wiederum verdampft, mit etwas Wasser versetzt und über Nacht stehen gelassen, wobei ausgeschiedene Kristalle abfiltriert wurden. Man erhielt 65 mg Oxalsäure, die nach Umkristallisieren aus Wasser bei 99,5—100,5°C schmolzen. Mischschmp. mit dem authentischen Präparat 100.5°C.

Anal. Gef.: C, 19,36; H, 5,04. Ber. für $C_2H_2O_4 \cdot 2H_2O$: C, 19,05; H, 4,80%.

Aus dem Filtrat wurden nach Behandeln mit basischem Bleiacetat und dann Acetylierung 63 mg Kristalle von Acetat erhalten. Nach 2-maligem Umkristallisieren aus heissem Alkohol ergaben sich farblose Kristalle vom Schmp. 167,5—168°C. Ein Mischschmp. mit dem synth. Präparat zeigte 169—169,5°C. Die IR-Spektren der zwei Präparate erwiesen sich als identisch.

Anal. Gef.: C, 50,07; H, 6,17. Ber. für $C_{25}H_{36}O_{17}$: C, 50,32; H, 5,85%.

Ozonspaltung des Narcissins: Eine Lösung von 200 mg Narcissin in 10 ccm 90 proz. Essigsäure wurde 80 Min. lang ozonisiert. Sie wurde dann im Vak. verdampft. Der Rückstand wurde wie oben beschrieben behandelt und der fast farblose Zucker betrug 60 mg nach dem Trocknen. Papierchromatographie wurde mit drei Lösungsmitteln (Pyridin, Butanol, Wasser 1:6:1; Eisessig, Butanol, Wasser 1:4:5; Essigester, Pyridin, Wasser 2:1:2)[2] durchgeführt. Nach Acetylierung wurden 39 mg rohe Kristalle erhalten. Dreimaliges Umkristallisieren gab farblose Kristalle vom Schmp. 167—168°C. Ein Mischschmp. mit dem synthetishen Produkt zeigte 168,5—169°C. Die IR-Spektren der beiden Präparate übereinstimmten. Zur Analyse wurde das Acetat nochmals umkristallisiert; Schmp. 169—169,5°C.

Anal. Gef.: C, 50,44; H, 6,15. Ber. für $C_{25}H_{36}O_{17}$: C, 50,32; H, 5,85%.

Bei der Ausführung der Synthese arbeitete Herr Toshiaki Miyajima mit.

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4) M. Kotake und H. Arakawa, *Naturwiss.*, **43**, 327 (1956); H. Arakawa, *J. Chem. Soc. Japan*, **77**, 1057 (1956). Nochmals sammelten wir den Pollen.
5) G. Zemplén und A. Gerces, *Ber.*, **67**, 2049 (1934).
6) G. Zemplén und A. Gerces, *Ber.*, **68**, 1318 (1935).
7) Herrn Prof. T. Kubota sei für die freundliche Uberlassung des Präparates gedankt.
** Vgl. F. A. H. Rice und L. C. Browning, *Science*, **125**, 496 (1957).

The Composition of Strong Phosphoric Acids

By Shigeru OHASHI and Hiroko SUGATANI

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Since several years ago new analytical methods[1] for the determination of carbon, nitrogen, sulfur and so on by the use of strong phosphoric acids as reaction media have been investigated in the authors' laboratory and a preliminary study on the composition of strong phosphoric acids was already reported[2].

The composition of strong phosphoric acids having 72 to 89 wt. % phosphorus(V) oxide was studied by Bell[3] who employed the so-called zinc titration method for the analysis of pyro- and tripolyphosphate. Thereafter many analytical methods were developed for the determination of various types of condensed phosphate; filterpaper chromatography[4], anion-exchange chromatography[5], pH-titration method[6], X-ray diffractometry[7], isotope dilution method[8], cobaltcomplex method[9], and infrared absorption spectrometry[10].

Higgins and Baldwin[11] used the anion-exchange chromatography, which was presented by Beukenkamp, Rieman III, and Lindenbaum[5], for their study on the composition of strong phosphoric acids which were prepared by dehydration of 85 wt. % orthophosphoric acid at the constant temperatures, 100° and 176°C. During the course of the present work Huhti and Gartaganis[12] published their report on the composition of strong phosphoric acids having 68.8 to 86.3 wt. % phosphorus (V) oxide, which were analyzed by the method of filterpaper chromatography.

In the present paper the composition of strong phosphoric acids containing about 67 to 80 wt. % phosphorus (V) oxide, which were prepared from 85 wt. % orthophosphoric acid under various conditions, was investigated in detail by the use of the anion-exchange chromatography[5] combined with the usual molybdenum-blue colorimetric analysis for orthophosphate.

Experimental

Preparation of Strong Phosphoric Acids.— About 30 g. of 85 wt. % orthophosphoric acid was heated on an electric heater as shown in Fig. 1 until a thermometer immersed in the acid indicated a definite temperature, 161°, 183°, 203°, 220°, 244°, 260°, 280°, 301°, 340°, or 380°C, respectively (sample Nos. 1 to 10 in Table I). The

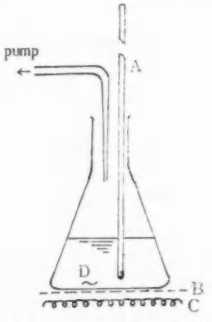

Fig. 1. Dehydration of orthophosphoric acid.
A: Thermometer C: Electric Heater
B: Wire Gauze D: Platinum Wire

time required for the dehydration was eight, sixteen, twenty five, thirty one, and sixty minutes for the temperatures, 183°, 220°, 301°, 340°, and 380°C, respectively. In a similar way about 30g. and 300 g. of 85 wt. % orthophosphoric acid were

1) T. Kiba, S. Ohashi, T. Takagi and Y. Hirose, *Japan Analyst*, **2**, 446 (1953); S. Ohashi, This Bulletin, **28**, 171, 177, 537, 585 and 645 (1955); T. Kiba, T. Takagi, Y. Yoshimura and I. Kishi, ibid., **28**, 641 (1955); T. Takagi, *Japan Analyst*, **4**, 624 (1955); S. Ohashi and H. Makishima, This Bulletin, **29**, 700 (1956); T. Kiba and I. Kishi, ibid., **30**, 44 (1957); S. Takagi and N. Hayashi, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **78**, 445 (1957).

2) S. Ohashi, This Bulletin, **28**, 537 (1955).

3) R. N. Bell, *Ind. Eng. Chem.*, **40**, 1464 (1948); cf. R. N. Bell, A. R. Wreath and W. T. Curless, *Anal. Chem.*, **24**, 1997 (1952).

4) J. P. Ebel et al., *Compt. rend.*, **233**, 415 (1951), **242**, 647 (1956) and *Bull. Soc. Chim. France*, **1953**, 991, 998, 1089 and 1096; J. P. Crowther, *Anal. Chem.*, **26**, 1383 (1954); E. Karl-Kroupa, ibid., **28**, 1091 (1956).

5) J. Beukenkamp, W. Rieman III and S. Lindenbaum, ibid., **26**, 505 (1954); S. Lindenbaum, T. V. Peters, Jr., and W. Rieman III, *Anal. Chim. Acta*, **11**, 530 (1954).

6) J. R. Van Wazer, E. J. Griffith and J. F. McCullough, *Anal. Chem.*, **26**, 1755 (1954).

7) A. J. Mabis and O. T. Quimby, ibid., **25**, 1814 (1953).

8) O. T. Quimby, A. J. Mabis and H. W. Lampe, ibid., **26**, 661 (1954).

9) H. W. McCune and G. J. Arquette, ibid., **27**, 401 (1955).

10) D. E. C. Corbridge and E. J. Lowe, ibid., **27**, 1383 (1955).

11) C. E. Higgins and W. H. Baldwin, ibid., **27**, 1780 (1955).

12) A. L. Huhti and P. A. Gartaganis, *Can. J. Chem.*, **34**, 785 (1956).

TABLE I
COMPOSITION OF STRONG PHOSPHORIC ACIDS

Sample No.	Initial Weight g.	Temp. °C	Specific Gravity at 15°C	Total P_2O_5 wt. %	Ortho H_3PO_4 wt. %	Pyro $H_4P_2O_7$ wt. %	Tripoly $H_5P_3O_{10}$ wt. %	Tetrapoly $H_6P_4O_{13}$ wt. %	Water H_2O wt. %
1	25.68	161	1.77	62.5	86.3	—	—	—	13.7
2	26.37	183	1.81	66.7	90.9	1.1	—	—	8.0
3	28.63	203	1.84	68.5	89.9	4.4	—	—	5.8
4	29.28	220	1.87	68.9	90.5	4.2	—	—	5.3
5	28.14	244	1.89	69.2	90.0	5.0	—	—	5.0
6	28.92	260	1.90	71.8	89.8	8.1	0.3	—	1.8
7	28.79	280	1.92	75.1	66.1	29.3	4.6	—	—
8	29.24	301	1.95	76.2	53.8	39.5	6.8	—	—
9	33.52	340	2.03	78.3	29.4	47.4	22.7	0.5	—
10	32.06	380	2.08	80.1	16.7	38.6	24.8	19.9	—
11	30	300	1.99	78.4	29.8	45.4	20.4	4.4	—
12	300	301	2.07	77.2	44.4	39.8	12.3	3.6	—
13	10.70	140[a]	1.92	74.5	74.1	23.5	2.5	—	—
14	10	140[a]	1.93	74.7	69.4	28.2	2.1	0.3	—

a) at the constant temperature, 140°±2°C.

dehydrated by raising the temperature of the liquid up to 300°C for sixty minutes (samples Nos. 11 and 12 in Table I). The latter is the so-called "300°C-strong phosphoric acid", which has been used for many analytical purposes in the authors' laboratory. There was no apparent attack on the glass in the preparation described above.

On the other hand about 10 g. of 85 wt. % orthophosphoric acid was taken in a platinum crucible and dehydrated by heating in a constant temperature oven at 140°±2°C for twenty one and thirty one hours, respectively (sample Nos. 13 and 14 in Table I).

Specific Gravities of the Strong Phosphoric Acids were measured using a 1-ml. or 2.5-ml. specific gravity bottle at 15°C.

Analysis of the Strong Phosphoric Acids.—A 0.2 to 0.8 g. portion of the strong phosphoric acid was weighed in a weighing tube, dissolved in 30 ml. of distilled water in 100-ml. volumetric flask, immediately neutralized to phenolphthalein with 1 N sodium hydroxide and the volume adjusted to the mark with distilled water. An aliquot of this solution, 2 ml. was poured into a column filled with resin. Anion exchanger, Dowex 1 was used under the following conditions: 120 to 200 mesh, 8% cross-linked, a column of 0.79 sq. cm. ×20 cm., exchange capacity of 2.28 meq./g., 2.62 g. in the dry state, chloride form. Elutions of individual phosphates were made by the method of Higgins and Baldwin[11]. Using a flow rate of 0.5 to 0.7 ml./min., orthophosphate was removed with 0.05 M hydrochloric acid, pyrophosphate with 0.20 M potassium chloride, tripolyphosphate with 0.27 to 0.35 M potassium chloride, and tetrapolyphosphate with 0.35 to 0.50 M potassium chloride.

By the first run the phosphate contained in each fraction of 5 ml. of eluate solution was determined colorimetrically by means of Nakamura's molybdenum-blue method[13]. Although orthophosphate was directly determined by the colorimetry, polyphosphates were hydrolyzed by heating with diluted sulfuric acid to orthophosphate before the colorimetric determination. A typical elution curve is shown in Fig. 2. In the second run, after the resin was again loaded with the same sample solution, the quantity of each eluant solution, required to separate the phosphate mixture, was let flow through the column, collected in a volumetric flask, and diluted to the mark with distilled water. An appropriate aliquot of the solution, after being hydrolyzed and concentrated, if necessary, was subjected to determination of phosphorus as previously described.

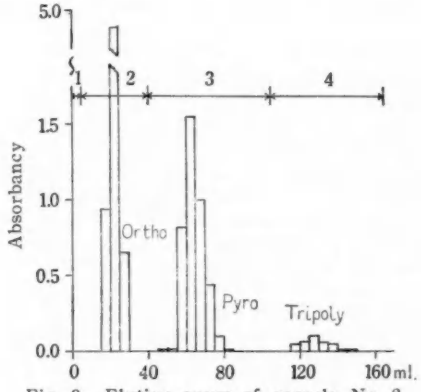

Fig. 2. Elution curve of sample No. 8.
1: Sample soln. and water
2: 0.05 M HCl
3: 0.20 M KCl
4: 0.35 M KCl

13) M. Nakamura, *J. Agr. Chem. Soc. Japan*, **24**, 1 (1950).

Results and Discussion

The results of the analyses of the strong phosphoric acids are shown in Table I. This indicates that the formation of pyro-, tripoly- and tetrapolyphosphoric acid starts at the concentration of phosphorus (V) oxide, around 67, 72 and 75 wt. %, respectively. The so-called "300°C-strong phosphoric acid" in the authors' laboratory, the sample No. 12, is composed of 44.4 wt. % orthophosphoric acid, 39.8 wt. % pyrophosphoric acid, 12.3 wt. % tripolyphosphoric acid, and 3.6 wt. % tetrapolyphosphoric acid. However, even the strong phosphoric acids which were prepared by heating orthophosphoric acid up to the same temperature, 300°C, have quite different compositions depending upon the weight of orthophosphoric acid taken and the elevation rate of the temperature. These facts may be clarified by comparing the results of the sample Nos. 8, 11 and 12.

Higgins and Baldwin[11] heated orthophosphoric acid at the constant temperature, 176°C for sixteen hours and obtained a mixture of 51 wt. % orthophosphoric acid, 38 wt. % pyrophosphoric acid, 9 wt. % tripolyphosphoric acid, 2 wt. % tetrapolyphosphoric acid, and 0.2 wt. % pentapolyphosphoric acid. No change in the composition was noted on prolonged heating up to 32.5 hours at 176°C. Therefore, the difference between the composition of the sample Nos. 13 and 14 in Table I, which were prepared by heating at 140°±2°C, may be due to the fluctuation of the temperature.

In Fig. 3 the relation between the composition of the strong phosphoric acids and their contents of phosphorus (V) oxide are illustrated. Generally they coincide with the data obtained by Bell[3], Higgins and Baldwin[11], and Huhti and Gartaganis[12], even if the strong phosphoric acids were prepared under different conditions. As indicated by Bell[3] and Huhti and Gartaganis[12], this means that a dynamic equilibrium between the component acids is set up, when the strong phosphoric acids are prepared by heating. However, Bell's data in higher phosphorus (V) oxide content may not be correct, because he did not determine separately the polyacid components higher than tripolyphosphoric acid. In the study by Huhti and Gartaganis small quantities of penta-, hexa- and heptapolyphosphoric acid were determined in the range from 78 to 80 wt. %

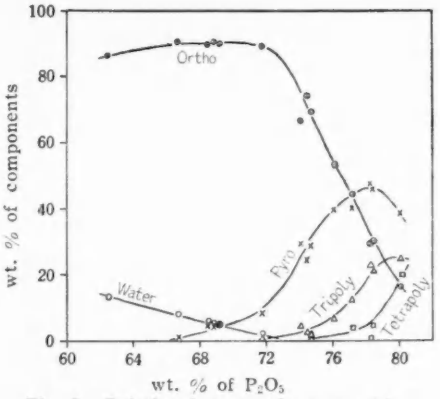

Fig. 3. Relation between the composition of the strong phosphoric acids and the contents of phosphorus (V) oxide.

phosphorus (V) oxide, but the present authors were not able to find them. In this respect the method of anion-exchange chromatography should be furthermore examined for the determination of higher polyphosphates.

During the course of the present investigation Parks and Van Wazer[14] published their treatise, in which a reorganization theory for the condensed phosphates as well as the strong phosphoric acids was developed. According to their theory for the strong phosphoric acids, in the general case, all five of the structural units, i. e. branching PO_4 units, middle PO_4 units, end PO_4 units, orthophosphoric acid units, and unreacted water molecule units may be present, with mole fractions b, m, e, o and u. All of the combinations whereby these units can interchange roles with each other are given by the following equations with the equilibrium constants.

$$2m=b+e \qquad K_1=b\cdot e/m^2 \qquad (1)$$

$$2e=m+o \qquad K_2=m\cdot o/e^2 \qquad (2)$$

$$2o=2e+u \qquad K_3'=(2e)\cdot u/o^2 \qquad (3)^{15}$$

In addition to the equations (1), (2) and (3), there are two other equations (4) and (5).

$$R=H_2O/P_2O_5=H/P$$
$$=(3o+2e+m+2u)/(b+m+e+o) \qquad (4)$$

$$b+m+e+o+u=1 \qquad (5)$$

14) J. R. Parks and J. R. Van Wazer, *J. Am. Chem. Soc.*, in press.

15) For this equilibrium another equilibrium constant $K_3=e^2\cdot u/o^2$ may be supposed, but the values of K_3 calculated from the experimental data were not constant in Parks and Van Wazer's study.

From the experimental data obtained by Huhti and Gartaganis[12] it was assumed that $K_1=1.0\times10^{-3}$, $K_2=8\times10^{-2}$, and $K_3'=2.0\times10^{-2}$ on a mole fraction basis. The theoretical curves of mole fractions of *b*, *m*, *e*, *o* and *u* were plotted as a function of *R* on the assumption mentioned above. These curves are shown in Fig. 4. The

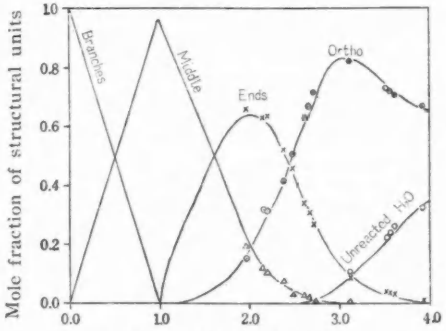

Fig. 4. Mole fractions of structural units as a function of H_2O/P_2O_5 mole ratio.

experimental data obtained by Huhti and Gartaganis agreed with the theoretically derived curves, although the data covered only the range from $R=1.2$ to $R=3.6$. The experimental data of the present study are shown as individual points in Fig. 4 and they also agree very well with the theory at least in the range from $R=2.0$ to $R=4.0$. This may prove the correctness of Parks and Van Wazer's theory.

Summary

The strong phosphoric acids in the range from 67 to 80 wt. % phosphorus(V) oxide were analyzed by the anion-exchange chromatography combined with the usual molybdenum-blue colorimetry. The so-called "300°C-strong phosphoric acid" which has been used for many analytical purposes in the authors' laboratory is composed of 44.4 wt. % orthophosphoric acid, 39.8 wt. % pyrophosphoric acid, 12.3 wt. % tripolyphosphoric acid, and 3.6 wt. % tetrapolyphosphoric acid. It was found that the relation of mole fractions of middle PO_4 units, end PO_4 units, orthophosphoric acid units, and unreacted water molecule units versus the mole ratio H_2O/P_2O_5 agrees with the reorganization theory for the strong phosphoric acids, presented by Parks and Van Wazer.

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Oxidation of Chromia Supported on Alumina

By Yoshio MATSUNAGA

(Received May 25, 1957)

Since 1946 Selwood and his coworkers[1] have carried out a detailed study of the magnetic properties of certain catalysts. By determining susceptibility-composition isotherms for a variety of supported oxides of transition elements, they deduced the oxidation state, the interaction covalence, and the dispersion of the paramagnetic ions. In the case of chromia on alumina[2], they concluded that, while the moment is nearly constant over the whole composition range, the change in susceptibility of chromium is almost entirely brought about by a change in the Weiss constant, namely the degree of dispersion. Selwood stated that the plus-three oxidation state of chromium is probably stabilized by the inductive action of the alumina support.

On the other hand, chromia has been known as a p-type semiconductor in an ordinary atmosphere and contains an excess of oxygen. The surface oxidation of chromia and chromia-alumina catalysts has been studied by many workers. Weller and Voltz[3] showed that the amount of excess of oxygen after treatment with oxygen at 500°C corresponds to the coverage of about one third of the total surface of the chromia. However, their study was concerned with only one commercial chromia-alumina catalyst, so it seems of interest to examine the correlation between the degree of dispersion and the stability of chromia towards oxidation at various temperatures.

Experimental

Preparation of Catalysts—The alumina and the chromia-alumina catalyst used in this study were prepared by a method similar to that described by Eischens and Selwood. Aluminum was dissolved in a ten per cent. solution of potassium hydroxide, then precipitated by acidification with a stream of carbon dioxide. The washed precipitate was dried and calcined at 600°C for twelve hours. The product was identified as gamma-alumina by X-ray diffraction. Alpha-alumina was prepared by further heat-treatment at about 1100°C for two weeks. The magnetic susceptibilities were found to be -0.37×10^{-6} for gamma-alumina and -0.33×10^{-6} for alpha-alumina. These values were independent of the field strength.

Catalyst samples were prepared by the impregnation method with chromic acid solution. By modifying the concentration of the chromic acid solution, catalysts with chromium concentration from about 1 to 33 per cent. were obtained. Samples were dried for eight hours at 350°C and reduced in a stream of hydrogen for five hours at 360°C. Analysis of each catalyst sample was carried out as follows. The samples were dissolved in hot concentrated sulfuric acid and diluted. The chromic ion was oxidized with potassium persulfate using silver nitrate as catalyst and the excess of the oxidant was decomposed by boiling the solution. After cooling, an excess of ferrous ammonium sulfate was added and the solution was titrated with standard dichromate solution, using diphenylamine in concentrated sulfuric acid as indicator.

Oxygen Treatment—The reduced catalysts were cooled in a stream of hydrogen and oxidized with air at room temperature. Further oxidations were carried out in a stream of oxygen for five hours at 250, 350 and 450°C.

Determination of Oxidation Number of Chromium—An iodometric method was employed for this purpose as described by Weller and Voltz[3]. A weighed amount from 100 to 300 milligrams of the sample was placed in about 30 cc. of distilled water and to it were added about two grams of potassium iodide and 10 cc. of distilled hydrochloric acid. The mixture was allowed to stand for about thirty minutes and the suspension was titrated with standard sodium thiosulfate solution (0.02N) with the use of starch solution as indicator. The mean oxidation number of chromium was calculated on the assumption that chromic ions equivalent to the active oxygen are present in a valent state higher than three.

Magnetic Measurement—The susceptibilities were measured at room temperature using a Gouy balance as previously described[4]. The field was calibrated at two constant current strengths with distilled water. When samples exhibited field strength dependence of susceptibility,

1) P. W. Selwood, "Catalysis" vol. 1 ed. by P. H. Emmett, Reinhold Pub. Corp., New York, 1954, p. 353.
2) R. P. Eischens and P. W. Selwood, *J. Am. Chem. Soc.*, **69**, 1590, 2698 (1947).
3) S. W. Weller and S. E. Voltz, ibid., **76**, 4695, 4701 (1954) and references cited there.
4) H. Akamatu and Y. Matsunaga, This Bulletin, **26**, 364 (1953).

the ferromagnetic effect was calibrated by the formula[5],

$$\chi_H = \chi + 2\sigma c/H$$

where χ_H is the apparent susceptibility observed at the field strength H, σ the saturation magnetization of the ferromagnetic impurity, c its concentration, and χ the susceptibility free from ferromagnetism.

The gram susceptibility of chromium was evaluated on the assumption that the susceptibility contributions of chromium, aluminium, and oxygen ions are additive, and those of the last two are independent of concentration.

Results

The first series to be considered is that in which gamma-alumina is used as a carrier. Fig. 1 shows the apparent oxidation number of chromium, determined by the chemical analysis, plotted against the concentration of chromium. The specific susceptibilities of chromium derived by direct application of the Wiedemann additivity law from the values per gram of catalyst samples are conveniently shown graphically in Fig. 2. Eischens and Selwood chose to call such curves "susceptibility-composition isotherms". Similar data for the second series, in which chromic acid is impregnated on alpha-alumina, are shown in Figs. 5 and 6.

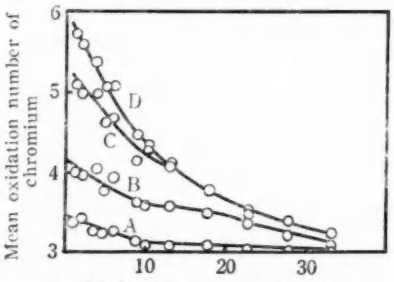

Fig. 1. Relation of oxidation number of chromium supported on gamma-alumina to its concentration: A, oxidized at room temperature; B, at 250°C; C, at 350°C; D at 450°C.

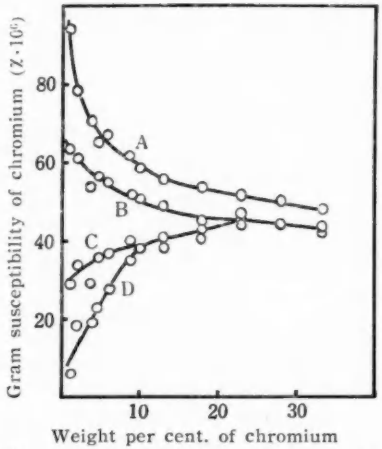

Fig. 2. Susceptibility-composition isotherms for chromia impregnated on gamma-alumina: A, oxidized at room temperature; B, at 250°C; C, at 350°C; D, at 450°C.

Discussion

The reduced catalyst samples are green or bluish white in a stream of hydrogen, but those of low concentration of chromium change their color to grayish white or grayish green in a short time after contact with air. These color changes suggest that the oxidation proceeds perceptibly at room temperature and, as the concentration of chromium decreases, air reacts with the chromic ion more rapidly. By direct chemical analysis, chromium in an oxidation state higher than three was detected over the whole concentration range. As shown in Fig. 1 curve A, the mean oxidation number is appreciably higher than three in the catalyst samples which contain less than ten per cent. of chromium and the limiting value at infinite dilution seems to be about 3.5.

When the temperature of oxygen treatment is raised above 250°C, the catalyst samples in the concentration range of less than eight per cent. change their color to yellowish white and those in the range from eight to twenty per cent. show grayish brown color. These remarkable color changes indicate that a drastic increase in oxidation number of chromium is likely to occur during oxygen treatment at such high temperatures and the results of chemical analysis confirm this view. In the limiting case, infinite dilution of chromium, the oxidation number increases until it finally reaches six, namely chromia is oxidized completely to chromic anhydride. The oxidation of chromia found here is in sharp contrast to our knowledge of the thermal decomposition of chromic anhydride. Glemser and his coworkers[6] showed that chromic

5) L. F. Bates, "Modern Magnetism" Cambridge Univ. Press, 1951, p. 135.

6) O. Glemser, U. Hauschild and F. Trüpel, *Z. anorg. allgem. Chem.*, **277**, 113 (1954).

anhydride begins to decompose in a stream of oxygen at about 250°C, the products being $CrO_{2.6}$ at temperature range from 283 to 314°C, $CrO_{2.4-5}$ from 334 to 388°C, and $CrO_{1.6}$ above 400°C. Therefore, the remarkable oxidation of supported chromia can not be attributed to a gross change in crystal nuclei, but the large surface area due to their dispersed structure may be responsible. It appears that the stabilization of the plus-three state of chromium by the inductive action of the alumina support is not effective enough for surface oxidation of chromia to be appreciably prevented.

To elucidate the shapes of the curves in Fig. 1, we must refer to the susceptibility isotherm method developed by Eischens and Selwood; this method revealed the dispersed structure of supported chromia from the magnetic data. Eischens and Selwood established that the susceptibility of chromium in supported chromia follows the Curie-Weiss law, $\chi = C/(T+\Delta)$, and noticed that the Curie constant C which relates to the oxidation state of chromium is almost constant but that the Weiss constant Δ is markedly dependent on the chromium concentration. According to Heisenberg's theory, the Weiss constant may be related to the exchange effect between neighboring paramagnetic chromium ions by the formula,

$$\Delta = -2JzS(S+1)/3k,$$

where J is the exchange integral, z the number of equidistant paramagnetic neighboring ions, S the vector sum of spin moments, and k the Boltzmann constant. Eischens and Selwood calculated the relationship between z and the number of layers of chromia on the assumptions that the variation in the exchange integral can be ignored and also that supported chromia has the corundum structure consisting of a hexagonal close-packed oxide-ion lattice with chromic ions in two thirds of the octahedral holes. Their calculations are reproduced graphically in Fig. 3. They pointed out that the graph of z against the number of layers changes its direction fairly sharply at about three layers and concluded that at the point where the susceptibility-composition isotherm changes its direction, the crystal nuclei of chromia spread on gamma-alumina are, on the average, three layers thick. In our results shown in Fig. 2 curve A, such a characteristic point which was called "point 1" is found at about ten per cent. of chromium. In the higher concentration range of chromium, these crystal nuclei tend to grow and to thicken. On the other hand, as the concentration of chromium decreases, the crystal nuclei shrink and, at the limit, chromium ions may be in two-dimensional atomic dispersion.

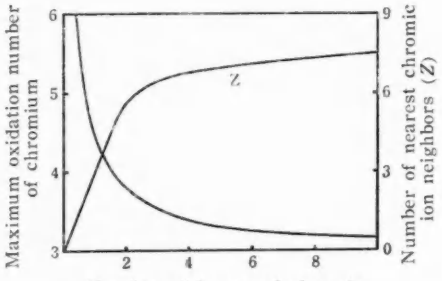

Fig. 3. Relation of maximum oxidation number of chromium and number of nearest chromic ion neighbors to number of layers of chromia.

It must be noted that the oxidation of chromia is especially marked in the concentration range of chromium less than that corresponding to point 1. The decrease in gram susceptibility of chromium means an increase of chromium in a valence state higher than three. Chromium ions in the plus-four and five states are less paramagnetic than those in the plus-three state and susceptibility of chromic acid may be nearly zero; therefore, the shapes of the susceptibility isotherms for the catalyst samples oxidized at higher temperatures (curves B, C and D in Fig. 2) are not only consistent with the above-mentioned view, but also show such a tendency more clearly. A chromium ion in the interior of crystalline chromia is completely surrounded by oxide ions and can not be oxidized directly with oxygen unless it migrates to the surface. But a chromic ion situated on the surface has not a sufficient number of oxide ions to enclose it and there is a possibility of suffering oxidation. The relationship between z and the number of chromia

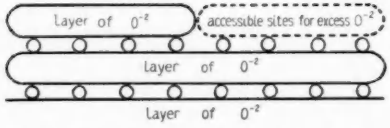

Fig. 4. Schematic representation of two layers of chromia, ○ Cr^{+3}.

layers is determined by the relative importance of the surface layer, hence, we can conclude easily that the degree of possible oxidation stands in close relation with z.

We shall examine the expected relation more quantitatively. Fig. 4 is a very schematic representation of supported chromia which is just two layers thick. Electroneutrality can be achieved in a chromia nucleus when the number of chromic ions situated in the outermost layer is the same as that in the layer just below, and half of them are not covered with oxide ions as shown in the model. The vacant sites on the surface can accommodate excess oxygen atoms until half of the chromic ions in the outermost layer are oxidized completely to the sexavalent state. Therefore, if sufficient oxide ions are supplied into such a space in the outermost oxide layer, the increase in the mean oxidation number of chromium must be $(6-3)/2\times2$ as shown in Fig. 4. Similar examination of a crystal model shows that the maximum value of the mean oxidation number of chromium has the following values; for one layer, +4.50; for three layers, +3.50; for five layers, +3.30; and for ten layers, +3.15. The possible oxidation numbers thus calculated are shown graphically in Fig. 3 for the purpose of comparing with the relation between z and the number of layers of chromia. The magnitude of the calculated values seems to be rather small. The disaccordance may be due to the existence of defects in the interior of the crystal which we ignored in the above examination. Some of them may contribute to oxidation directly and others may promote the migration of ions to the surface and create a further possibility of oxidation. Therefore, it is not too much to say that the possibility of surface oxidation explains satisfactorily the remarkable observed oxidation of supported chromia.

In the second series, the position of point 1 in the susceptibility isotherm for the catalyst samples oxidized at room temperature (Fig. 6 curve A) is not clear. However, by analogy to the first series, we can easily locate it in the isotherms for the catalysts oxidized at higher temperatures (Fig. 6 curves C and D) and also in the relations between the mean oxidation number and the concentration of chromium shown in Fig. 5. Point 1 is found at about two or three per cent. of chromium and its shift to a lower concentration of

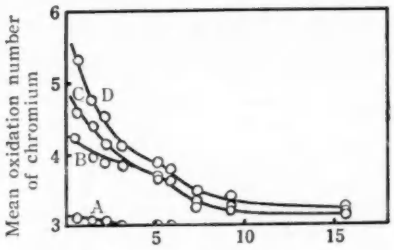

Fig. 5. Relation of oxidation number of chromium supported on alpha-alumina to its concentration: A, oxidized at room temperature; B, at 250°C; C, at 350°C; D, at 450°C.

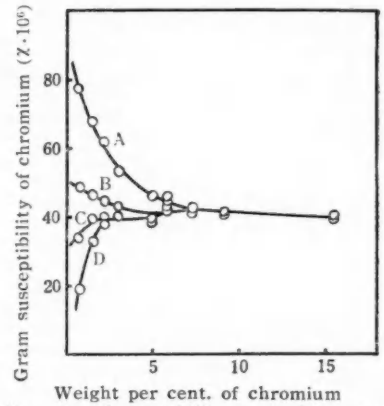

Fig. 6. Susceptibility-composition isotherms for chromia impregnated on alpha-alumina: A, oxidized at room temperature; B, at 250°C; C, at 350°C; D, at 450°C.

chromium may be due to a decrease in surface area of the carrier.

The susceptibility isotherms C and D in both the first and the second series show the existence of a characteristic point where the concentration of chromium is about twice that at point 1. The magnetic susceptibility of chromium in the catalyst samples oxidized at 350 and 450°C is nearly constant between these two points. At present the author can not give any explanation to this new point.

Although point 1 was shown at first in the susceptibility isotherm by Eischens and Selwood and its meaning was given on the basis of the magnitude of Weiss constant as clarified in the preceeding paragraphs, its position in the chromia-alumina catalyst system can be easily pointed out in the relation between the

mean oxidation number and the concentration of chromium in the samples oxidized at the temperature higher than 350°C. The latter method may be more convenient than the magnetic method as it needs only the apparatus for the usual chemical analysis. Besides, at least in this catalyst system our chemical method has the possibility of giving a measure of the surface area of promoter. It is not yet clear whether or not the application of the method described here is limited to this case.

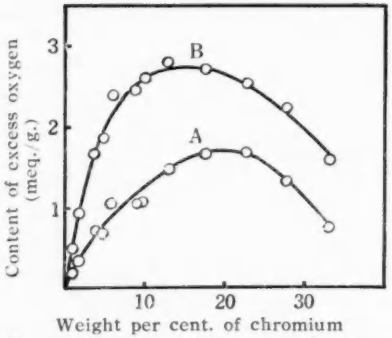

Fig. 7. Relation of content of excess oxygen in chromia-gamma-alumina catalysts to chromium concentration: A, oxidized at 250°C; B, at 450°C.

As the oxidation of supported chromia can be closely related to its high surface area, if we plot the content of excessive oxygen in one gram of the oxidized catalyst sample against the concentration of chromium, the composition at which the surface area of chromia per gram of catalyst reaches its maximum value will be indicated. As shown in Fig. 7, the catalysts which contain from 15 to 20 per cent. of chromium have the greatest values of excessive oxygen. Therefore, when we need to prepare the chromia-alumina catalyst in which the surface area of chromia is as high as possible, we must impregnate chromic acid solution on gamma-alumina, as the final concentration of chromium will be from 1.5 to 2 times that corresponding to point 1.

A similar study on the chromia-zinc oxide catalyst system was also made. The results will be presented in a later paper.

Summary

Oxidation of chromia supported on gamma- and alpha-alumina at room temperature, 250, 350 and 450°C was examined by chemical and magnetic method. The mean oxidation number of chromium in the oxidized catalyst samples was determined as a function of the concentration of chromium. In the extreme case, infinite two-dimensional dilution of chromium, it was shown that chromia is oxidized completely to chromic anhydride. This chemical change is in sharp contrast to our knowledge of the thermal decomposition of chromic anhydride. The remarkable property of supported chromia to suffer oxidation was attributed to the relative importance of surface oxidation and explained nearly quantitatively by comparing it with the conclusion deduced from the susceptibility-composition isotherm method developed by Selwood and his coworkers. Therefore, the study of oxidation of the chromia-alumina catalyst system by chemical analysis seems to be useful to the same degree as the magnetic method proposed by Selwood and more convenient for deducing the dispersed structure of supported chromia. Finally the condition of preparing the catalyst sample which has the greatest surface area of chromia per gram of catalyst was examined in reference to the above-mentioned conclusion.

The author wishes to express his hearty thanks to Professor H. Akamatu for his kind direction.

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*Reaction Rate and Electron Transfer Mechanism in Disproportionation Reaction of Uranium (V)**

By Hideo IMAI

(Received May 8, 1957)

In acid solution, sexavalent uranium (UO_2^{++}) is reduced to unstable quinqulvalent uranium at the dropping mercury electrode, and is regenerated by the disproportionation reaction of the reductant (U(V)) as is schematically shown in the following equations:

$$U(VI)+e=U(V): \text{ Electrode Reaction (1)}$$

$$U(V)+U(V)=U(VI)+U(IV): \text{ Disproportionation Reaction (2)}$$

Since both the sexavalent uranium diffused from the bulk solution and the one regenerated by the disproportionation reaction reach the electrode and are reduced, the limiting current has a reaction-controlled as well as a diffusion-controlled component[1]. The increase in the current beyond that corresponding to a one-electron diffusion-controlled reduction is a function of the rate of the disproportionation, and one can estimate the rate constant from the measurement of the limiting current.

Hitherto, it has been found that the rate linearly increases with the hydrogen ion concentration[2] and it increases in the presence of chloride[3]. With regard to this effect of chloride it was assumed that the increment in the rate may be assigned to the ζ-potential of the electrode surface.

In this connection, the increase in the rate of some isotopic electron exchange reactions such as Fe(II)—Fe(III)[4], Hg(I)—Hg(II)[5], Ce(III)—Ce(IV)[6], Eu(II)—Eu(III)[7] and Cr(II)—Cr(III)[8] has been observed in homogeneous solutions containing chloride. The present author has also found that the electron transfer of some electrode processes such as the electrolytic reduction of thallous, cadmium and zinc ions under the continuously changing potential is facilitated in solutions containing sulfate, chloride, bromide and iodide in the order given[9]. In this paper a similar effect of various anions on the disproportionation rate process of uranium (V) is investigated, and a tentative reaction mechanism presented.

Experimental

Apparatus.—The current-voltage curves were recorded with a SHIMADZU Model SH-2 photographically recording polarograph, whose maximum current-sensitivity was 2.85×10^{-9} amp./mm./m. The cell used was an H type with triple sintered glass partitions. In two compartments between the test solution and the saturated calomel electrode, the saturated solution of sodium perchlorate and that of sodium chloride were instilled in order to avoid the formation of insoluble potassium perchlorate and also to avoid the contamination of the test solution by chloride. The construction was as follows:

(S.C.E.(Anode))—(Sat. NaCl)—(Sat. $NaClO_4$)—(Test solution-D. M. E. (Cathode)).

The temperature was kept constant within the error of ±0.1°C by a thermostat. With a head of 30 cm. of mercury the mean life-time of the dropping mercury electrode in 2 f sodium perchlorate solution was 5.5_8 sec. at the applied voltage of -0.4 volts vs. S. C. E.

Reagent.—All the reagents were of analytical grade. To prepare the stock solution of uranyl perchlorate, uranyl acetate ($UO_2(C_2H_3O_2)_2\cdot 2H_2O$) was evaporated three times with perchloric acid and dissolved in 2 f perchloric acid solution. The test solutions were prepared by diluting this with supporting electrolyte solutions.

The concentration of uranyl ions was determined as follows. Ammonium hydroxide was added to 10 ml. test solution and, after digestion

* Presented at the Discussion on Polarography held by the Electrochemical Society of Japan in November, 1956, and the 10th Annual Meeting of the Chemical Society of Japan held in April, 1957.

1) I. M. Kolthoff and W. E. Harris, *J. Am. Chem. Soc.*, **67**, 1484 (1945); **68**, 1175 (1946).
2) E. F. Orlemann and D. M. Kern, ibid., **75**, 3059 (1953).
3) J. Koryta and J. Koutecky, *Collection Czechoslov. Chem. Communs.*, **20**, 423 (1955).
4) J. Silverman and R. W. Dodson, *J. Phys. Chem.*, **56**, 846 (1952).
5) R, L. Wolfgang and R. W. Dodson, ibid., **56**, 872 (1952).
6) H. C. Hornig and W. F. Libby, ibid., **56**, 869 (1952).
7) D. J. Meier and C. S. Garner, ibid., **56**, 853 (1952).
8) H. Taube and H. Myers, *J. Am. Chem. Soc.*, **76**, 2103 (1954).
9) H. Imai and S. Chaki, This Bulletin, **29**, 498 (1953).

for thirty minutes on a water bath, the precipitate was filtered on a No. 7 Tōyō filter paper. Then, the precipitate was washed with water containing ammonium hydroxide and ignited to red heat. The residue was weighed as U_3O_8.

The concentration of perchloric acid in the test solution was determined by titration with the standard solution of sodium hydroxide. Since the basic salt of uranyl perchlorate begins to precipitate at pH greater than 4, methyl orange is qualified for the indicator. The ionic strength was kept at a constant value of 2.1 f by adding sodium perchlorate. In all cases no surface active reagent was used, since it reduced the reaction rate. Nitrogen gas purified by van C. Brunt's method was used to remove the dissolved oxygen in the test solution prior to submission to polarography.

Current-Voltage Curve.—The current-voltage curves of uranyl in various supporting electrolytes are shown in Fig. 1. Uranyl developed well-defined polarographic waves in 2 f sodium perchorate solution, there being no polarographic maxima. The half-wave potential of the first wave was -0.17_0 volts vs. S. C. E., and that of the second -0.92_5 volts vs. S. C. E. The apparent

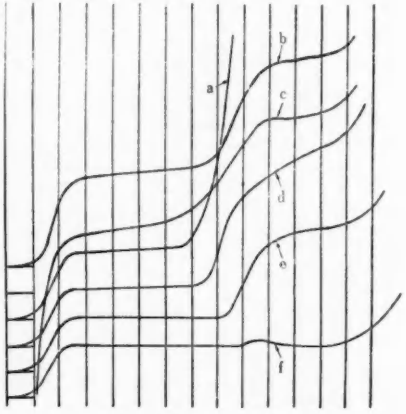


Fig. 1. Current-voltage curves of uranyl in various supporting electrolytes: Each curve starts at 0V vs. S. C. E. and each voltage interval corresponds to 0.1 V. A short horizontal line represents a galvanometer zero line. (UO_2^{2+}), 4.22 mf. Temp., 25°C. Ionic strength, 2.1 f.

a (1×10^{-1} f H^+, 2 f NO_3^-) b (1×10^{-1} f H^+, 2 f Cl^-)
c (1×10^{-1} f H^+, 2 f Br^-) d (1×10^{-1} f' H^+, 2 f ClO_4^-)
e (5×10^{-3} f H^+, 2 f ClO_4^-) f (2×10^{-3} f H^+, 2 f ClO_4^-)

number of electrons transferred per ion was one in the case of the first reduction, and two in the case of the second reduction. When the hydrogen-ion concentration was less than 10^{-3} f, the second wave disappeared, while the first wave decreased to a definite height, giving a pure diffusion-controlled current. The first reduction gives quinquevalent uranium, which is unstable in acid solution and disproportionates into sexavalent uranium and quadrivalent uranium. The quinquevalent uranium is reduced to tervalent uranium in the second reduction. It is assumed that in a slightly acidic solution the basic salt of quinquevalent uranium precipitates, and accordingly, the second wave disappears.

Estimation of the Rate Constant.—The disproportionation reaction (equation 2) is of the second order with respect to quinquevalent uranium. Accordingly, the rate of the regeneration of uranium (VI) is given as follows;

$$dU(VI)/dt = 1/2 \cdot kU(V)^2 \tag{3}$$

where k is the rate constant.

This flux of the sexavalent uranium contributes to the first reduction as well as the flux of the pure diffusion. Hence, one arrives at the equations

$$\partial U(VI)/\partial t = D\partial^2 U(VI)/\partial x^2 + (2x/3t)\partial U(VI)/\partial x + 1/2 \cdot kU(V)^2 \tag{4}$$

$$\partial U(V)/\partial t = D\partial^2 U(V)/\partial x^2 + (2x/3t)\partial U(V)/\partial x - kU(V)^2 \tag{5}$$

the boundary condition being the same as in the case of a pure diffusion, i. e.,

$$\left.\begin{aligned} &t=0,\ x>0;\ U(VI)=a^*,\ U(V)=0 \\ &t>0,\ x=0;\ U(VI)=0,\ \partial U(VI)/\partial x = -\partial U(V)/\partial x \end{aligned}\right\} \tag{6}$$

where a^* is the concentration of uranyl in the bulk solution, and the diffusion constant (D) of the oxidant is assumed to be equal to that of the reductant.

The solution of equations 3 and 4 was given by Orlemann and Kern[2] by means of the concept of a reaction volume and was followed by Koutecky-Koryta's approximate solution[10] as is illustrated in the following;

$$\left.\begin{aligned} &\bar{i}_l/\bar{i}_d = 1 + \sum_{i=1}^{\infty} D_i \xi_1^i \\ &\xi_1 = 2a^* kt \end{aligned}\right\} \tag{7}$$

where $\bar{i}_l$ is the mean limiting current, $\bar{i}_d$ is the mean diffusion-controlled current, D_i is a numerical constant ($D_1=4.716\times10^{-2}$, $D_2=-4.679\times10^{-3}$, $D_3=5.350\times10^{-4}$, $D_4=-6.771\times10^{-5}$, ……) and t is the reaction time.

The pure diffusion-controlled current ($\bar{i}_d$) can be estimated in the solution containing about 10^{-3} f hydrogen ion, since in this solution the disproportionation reaction is virtually negligible. In this experiment $\bar{i}_l$ and $\bar{i}_d$ were measured at the applied voltage of -0.4 volts vs. S. C. E., where a plateau was given in the current-voltage curve, and t was the mean of the life-time of ten drops of the D. M. E. Thus, the rate constant was calculated and it was confirmed that, as far as the value of $\bar{i}_l/\bar{i}_d$ did not exceed 1.18, equation 7 gave sufficiently accurate values in comparison

10) J. Koutecky and J. Koryta, *Collection Czechoslov. Chem. Communs.*, **19**, 845 (1954).

TABLE I

EFFECT OF THE HYDROGEN ION ON THE DISPROPORTIONATION RATE CONSTANT

No.	(H^+) f	t sec.	$E_{1/2}$, V vs. S. C. E.	$\bar{i}_l$ μA	$\bar{i}_l/\bar{i}_d$	ξ	k $f^{-1}\cdot sec.^{-1}$	k_0 $f^{-2}\cdot sec.^{-1}$
1	0.002	5.5_8	-0.17_0	14.2	1	0	0	0
2	0.005	5.5_8	-0.17_0	14.2	1	0	0	0
3	0.03	5.5_8	-0.17_0	14.5	1.02_1	0.48	10	340
4	0.06	5.5_8	-0.17_0	14.9	1.04_9	1.16	25	410
5	0.09	5.5_8	-0.17_0	15.3	1.07_7	1.93	41	456
6	0.101_5	5.5_8	-0.17_0	15.4	1.08_5	2.19	47	458
7	0.12	5.5_8	-0.17_0	15.5	1.09_2	2.40	51	425
8	0.15	5.5_8	-0.17_0	15.7	1.10_6	2.86	61	405
9	0.18	5.5_8	-0.17_1	16.0	1.12_7	3.62	77	427
10	0.21	5.5_8	-0.17_1	16.3	1.14_8	4.43	94	448
11	0.24	5.5_8	-0.17_1	16.5	1.16_2	5.08	108	450
12	0.27	5.5_8	-0.17_2	16.8	1.18_3	—	—	—
13	0.30	5.5_8	-0.17_2	17.1	1.20_4	—	—	—
								$(436)_{mean}$

($a^* = 4.22\times10^{-3}$ f, $\mu = 2.1$ f ($NaClO_4$))

with those measured in homogeneous solutions[11]. The error for the estimation of the rate constant amounts to $\pm10\%$.

Results and Discussion

Effect of Hydrogen Ion. — Uranyl developed no well-defined wave in buffered media, so the acidity of the solution was controlled by perchloric acid. Strictly speaking the hydrogen ion concentration was not equal to the concentration at the electrode surface where the disproportionation reaction occurred. When there was a trace of the dissolved oxygen, the hydrogen ion concentration appreciably decreased at the electrode surface, owing to the formation of the hydroxide ion as the electrolytic reductant. Accordingly, the experiment was carried out after careful removal of the dissolved oxygen.

The hydrogen ion concentration, the limiting current and the rate constant are illustrated in Table I, and the rate constant is plotted against the hydrogen ion concentration in Fig. 2. The experimental conditions are appended to Table I.

From Table I and Fig. 2 one can conclude that the disproportionation reaction of equation 2 is of the first order with respect to the hydrogen ion; the rate constant (k_0) reduced to unit hydrogen ion concentration is given by

$$k_0 = k/(H^+). \qquad (8)$$

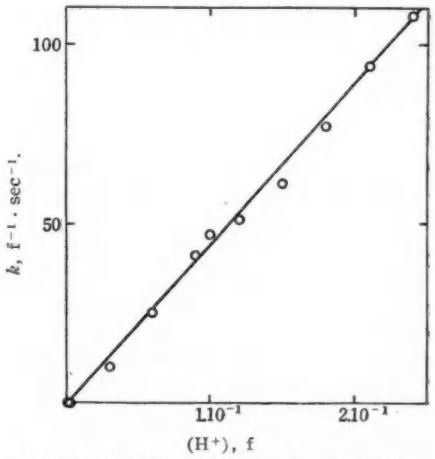


Fig. 2. Effect of the hydrogen ion on the disproportionation rate constant. Supporting electrolyte, 2.1 f $NaClO_4$; Temp., 25°C.

For convenience sake, the comparison of the rate constant will be referred to as k_0 in the following description.

Effect of Chloride.—The experimental conditions were as follows: the hydrogen ion concentration, $(H^+)=0.04_0$ f; the uranyl concentration, $a^*=1.95_4\times10^{-3}$ f; the reaction time $t=5.5_4$ sec.; the ionic strength, $\mu=2.04$ f; temperature, $T=298.2$°K.

Since the effect of chloride was multiplied by that of the hydrogen ion to yield too great an effect to bear on equation 7, a low concentration of the hydrogen ion was preferable to measure the effect in

11) D. M. Kern and E. F. Orlemann, *J. Am. Chem. Soc.*, **71**, 2102 (1949); K. A. Kraus et al., ibid., **71**, 2510 (1949); H. G. Heal and J. Thomas, *Trans. Faraday Soc.*, **45**, 11 (1949).

TABLE II
EFFECT OF CHLORIDE ON THE DISPROPORTIONATION RATE CONSTANT

No.	(Cl^-) f	t sec.	$E_{1/2}$, $V_{vs.}$ S. C. E.	$\bar{i}_l$ μA	$\bar{i}_l/\bar{i}_d$	ξ	k $f^{-1}\cdot sec.^{-1}$	k_0 $f^{-2}\cdot sec.^{-1}$
1	0	5.5_4	-0.17_0	7.8_2	1.01_9	0.42	19	475
2	0.2	5.5_4	-0.17_2	7.9_4	1.03_5	0.81	37	925
3	0.4	5.5_4	-0.17_4	8.0_1	1.04_3	1.01	48	1200
4	0.6	5.5_4	-0.17_5	8.0_8	1.05_2	1.12	52	1300
5	0.8	5.5_3	-0.17_8	8.1_1	1.05_6	1.34	62	1550
6	1.0	5.5_3	-0.18_0	8.1_3	1.05_9	1.42	66	1650
7	1.2	5.5_3	-0.18_4	8.2_1	1.06_9	1.70	79	1975
8	1.4	5.5_3	-0.18_7	8.2_8	1.07_8	1.96	91	2275
9	1.6	5.5_2	-0.19_1	8.4_4	1.09_9	2.62	121	3025
10	1.8	5.5_1	-0.19_3	8.4_7	1.10_3	2.76	128	3200
11	2.0	5.4_8	-0.19_5	8.5_4	1.11_2	3.08	149	3725

the wide range of the chloride concentration.

Under the above mentioned conditions, the pure diffusion-controlled current was 7.6_8 microamp., when the hydrogen ion concentration was 5.6×10^{-3} f.

The change in supporting electrolyte may result in the variation in the diffusion constant of a reducible species. Especially, this effect will increase, when a supporting electrolyte acts as a complexing agent. However, in this case the net variation of the diffusion constant is not noticeable; and furthermore, the diffusion-controlled current is proportional to the square root of the diffusion constant. Therefore, the diffusion rate will not play a leading role in the variation of the limiting current, while, it will be a cause of the error in the estimation of the rate constant. On the other hand, the increment of the ζ-potential of the electrode surface in the concentration region above 1 f is less than that in the concentration region below 1 f. Accordingly, the steady increase of the limiting current in the chloride concentration ranging from 0.2 to 2 f is mainly attributable to the increase in the rate constant.

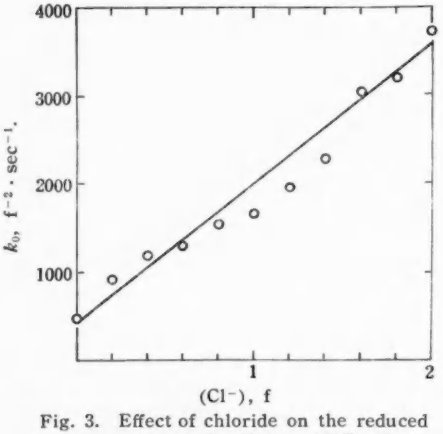


Fig. 3. Effect of chloride on the reduced rate constant, k_0. Temp., 25°C; Ionic strength, 2.1 f.

The results of this experiment are illustrated in Table II, and the rate constant is plotted against the chloride concentration in Fig. 3.

From these results it can be seen that the rate of the disproportionation reaction is of the first order with respect to chloride.

The variation of the half-wave potential with the chloride concentration indicates tacitly the existence of the uranyl chloride complex. In this case the kinetic current slightly affects the measurement of the half-wave potential and the mean discrepancy between the 1/4-wave potential and the 3/4-wave potential was about 62 millivolts, which was sufficiently close to the theoretically expected value of a reversible electrode process (59 millivolts)[12]. Hence, assuming that the stability constant of the complex does not vary in the high concentration region of the complexing agent, one can estimate the variation of the coordination number by the equation[13].

$$\bar{p}-\bar{q}=(\mathrm{d}(E_{1/2})_c/\mathrm{d}\log(X^-))\cdot(2.303RT/nF) \qquad (9)$$

where $\bar{p}$ and $\bar{q}$ are the mean coordination number of the higher oxidation state and that of the lower oxidation state, respectively. The plot of the logarithm of the

12) T. Thomas, *Collection Czechoslov. Chem. Communs.*, **9**, 12, 81, 150 (1937).
13) J. J. Lingane, *Chem. Revs.*, **29**, 1 (1941).

chloride concentration against the half-wave potential gives a straight line in the concentration region ranging from 1 f to 2 f as is illustrated in Fig. 4. The value of $\bar{p}-\bar{q}$ calculated from the tangent of the straight line was about unity.

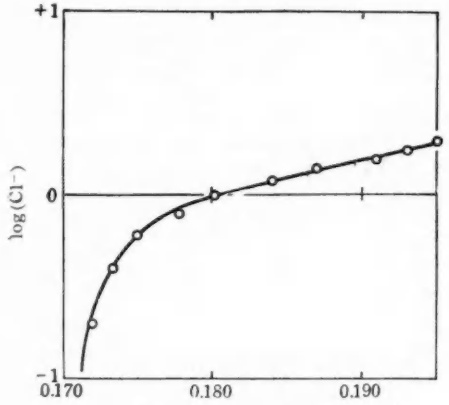


Fig. 4. Shift of the half-wave potential of the first reduction of uranyl by chloride. Temp., 25°C; Ionic strength, 2.1 f.

Effect of Various Anions. — By the author's datum the rate constant, k_0, was 436 $f^{-2} \cdot sec^{-1}$. in 2 f sodium perchlorate solution. By Koryta and Koutecky[3] it was 143 $f^{-2} \cdot sec^{-1}$. in 0.4 f sodium perchlorate, and was 700 $f^{-2} \cdot sec^{-1}$. in 3.8 f sodium perchlorate. From these data it can be seen that perchlorate appreciably affects the rate constant, while the effect of perchlorate was smallest in comparison with nitrate and bromide, and in particular, it was negligibly small in comparison with chloride and iodide. Accordingly, sodium perchlorate was qualified for keeping the ionic strength constant.

The experimental conditions were as follows: $(H^+)=0.101_5$ f; $a^*=4.22_5\times10^{-3}$ f; $\mu=2.1$ f; $T=298.2$°K.

The results are illustrated in Table III in which the life-time (t) of the D. M. E. appreciably changes with the bromide or the iodide concentration. Since the diffusion-controlled current is proportional to $t^{1/6}$ according to Ilkovic's equation, the variation in the life-time which amounts to 5% does not require any significant calibration for the pure diffusion-controlled current estimated in 2 f sodium perchlorate solution. The value of $\bar{\xi}$, however, is a linear function of t, hence, the variation of the reaction time was taken into account.

In Figs. 5 and 6 is plotted the anion concentration against the limiting current and the rate constant, respectively. In all cases the rate constant is a linear function of the anion concentration. This leads to the following equation;

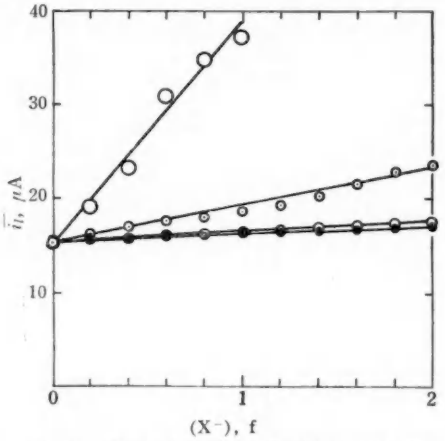


Fig. 5. Variation of the limiting current with the concentration of various anions. Temp., 25°C. Ionic strength, 2.1 f. (H^+), 0.101_5 f. ●···NO_3^-, ○···Br^-, •···Cl^-, ○···I^-.

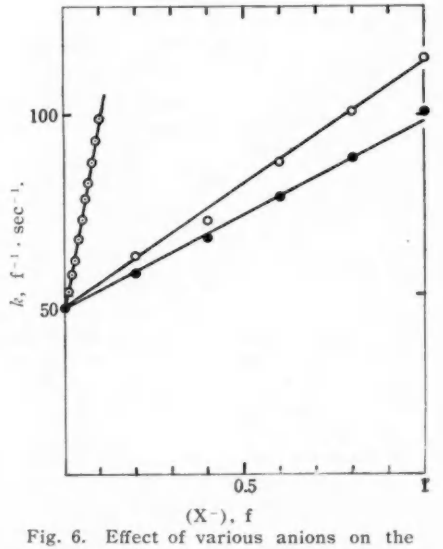


Fig. 6. Effect of various anions on the disproportionation rate constant. Temp., 25°C. Ionic strength, 2.1 f. ●···NO_3^-, ○···Br^-, ⊙···Cl^-.

TABLE III

COMPARISON OF THE EFFECT OF NITRATE, BROMIDE, CHLORIDE AND IODIDE ON THE DISPROPORTIONATION RATE CONSTANT

	No.	(X^-) f	t sec.	$\overline{i_l}$ μA	$\overline{i_l}/\overline{i_d}$	ξ	k $f^{-1}\cdot sec.^{-1}$	k_0 $f^{-2}\cdot sec.^{-1}$
NO_3^-	1	0	5.5_6	15.4	1.08_5	2.19	47	458
	2	0.2	5.5_6	15.6	1.09_9	2.63	56	550
	3	0.4	5.5_6	15.8	1.11_3	3.11	66	650
	4	0.6	5.5_6	16.0	1.12_7	3.62	77	758
	5	0.8	5.5_6	16.2	1.14_1	4.14	88	866
	6	1.0	5.5_5	16.4	1.15_5	4.75	101	995
	7	1.2	5.5_5	16.6	1.16_9	5.45	116	1143
	8	1.4	5.5_4	16.8	1.18_3	6.18	131	1291
	9	1.6	5.5_4	17.0	1.19_7	—	—	—
Br^-	1	0	5.5_8	15.4	1.08_5	2.19	47	458
	2	0.2	5.5_4	15.7	1.10_6	2.86	61	598
	3	0.4	5.5_0	15.9	1.12_0	3.35	71	700
	4	0.6	5.4_5	16.0	1.12_7	3.62	77	758
	5	0.8	5.4_1	16.4	1.15_5	4.75	101	995
	6	1.0	5.3_9	16.7	1.17_6	5.82	124	1222
	7	1.2	5.3_7	16.9	1.19_0	—	—	—
Cl^-	1	0	5.6_0	15.6	1.09_1	2.37	47	461
	2	0.04	5.6_9	15.8	1.10_5	2.83	56	551
	3	0.08	5.6_0	16.0	1.11_9	3.32	66	646
	4	0.12	5.6_0	16.3	1.13_6	3.96	78	770
	5	0.16	5.6_0	16.4	1.14_7	4.38	87	852
	6	0.20	5.6_0	16.6	1.16_1	5.04	99	973
	7	0.40	5.5_8	17.4	1.22_5	—	—	—
I^-	1	0	5.5_9	15.4	1.08_5	2.19	47	458
	2	0.2	4.7_5	19.1	1.34_5	—	—	—

TABLE IV

TEMPERATURE DEPENDENCE OF THE LIMITING CURRENT AND THE DISPROPORTIONATION RATE CONSTANT

	T °K	281	285	289	293	297	301	305	309
$\overline{i_d}$	t, sec.	5.8_0	5.7_4	5.6_8	5.6_4	5.6_0	5.5_6	5.5_2	5.4_9
	$\overline{i_d}$, μA	10.6	11.4	12.1	12.9	13.7	14.4	15.2	15.9
(H^+)	t, sec.	5.8_0	5.7_4	5.6_8	5.6_4	5.6_0	5.5_6	5.5_2	5.4_9
	$\overline{i_l}$, μA.	10.9	11.8	12.7	13.6	14.6	15.6	16.6	17.6
	$\overline{i_l}/\overline{i_d}$	1.02_8	1.03_5	1.05_0	1.05_4	1.06_6	1.08_3	1.09_2	1.10_7
	ξ	0.65	0.82	1.19	1.29	1.61	2.12	2.40	2.90
	k, $f^{-1}\cdot sec.^{-1}$	13	17	25	27	34	45	52	63
(H^+, Cl^-)	t, sec.	5.8_0	5.7_4	5.6_8	5.6_4	5.6_0	5.5_6	5.5_2	5.4_9
	$\overline{i_l}$, μA.	11.0	12.0	13.0	14.1	15.2	16.5	17.9	19.3
	$\overline{i_l}/\overline{i_d}$	1.03_8	1.05_3	1.07_4	1.09_3	1.10_9	1.14_6	1.17_8	1.21_4
	ξ	0.89	1.26	1.84	2.44	2.96	4.34	(5.92)	—
	k, $f^{-1}\cdot sec.^{-1}$	19	26	38	51	63	93	(127)	—
(H^+, Br^-)	t, sec.	5.5_9	5.5_4	5.5_0	5.4_6	5.4_3	5.4_0	5.3_7	5.3_5
	$\overline{i_l}$, μA.	11.1	12.1	13.2	14.4	15.7	17.1	18.6	20.3
	$\overline{i_l}/\overline{i_d}$	1.04_7	1.06_1	1.09_1	1.11_6	1.14_6	1.18_8	1.22_4	1.27_7
	ξ	1.11	1.47	2.37	3.21	4.34	(6.48)	—	—
	k, $f^{-1}\cdot sec.^{-1}$	24	31	51	70	95	(142)	—	—

($a^* = 4.22\times10^{-3}$ f)

$$dU(V)/dt = k_0' \cdot (X^-) \cdot U(V)^2 \qquad (10)$$

where (X^-) is the anion concentration. Thus, it can be concluded that the disproportionation reaction of uranium (V) is of the third order in this case. In view of the fact that the diffusion-controlled current of the cadmium ion increases in the presence of bromide and iodide, the increase in the limiting current of Table III can not be solely assigned to the increase in the disproportionation rate, but it may presumably include the effect of the ζ-potential. In this connection, it is noteworthy that a polarographic maximum appears in the test solution containing 1 f iodide. Furthermore, iodide was oxidized to iodine by perchloric acid in the test solution. Hence, the data in Table III are ill-defined in the cases of bromide and iodide.

The apparent half-wave potential was ill-defined in the solution containing iodide on account of the anodic current flowing at more negative potentials than the reduction potential of uranyl. Also, this effect exists in the case of bromide (cf. Fig. 1). However, it could be confirmed from the measurement in the solution containing low concentration bromide that the half-wave potential was slightly shifted by the presence of bromide. Similarly, the effect of nitrate was too small to deserve any attention**. However, the half-wave potential was appreciably shifted by chloride. It is noteworthy that a parallelism lies between the shift of the half-wave potential and the rate constant, except for the case of the hydrogen ion which does not affect the half-wave potential but increases the rate constant (cf. Table I).

From these results one can conclude that uranyl complex, whose change of the complexity is predominant in the course of the uranyl reduction, is liable to increase the rate of the disproportionation.

Temperature Dependence of the Rate Constant. — Test solutions containing 0.10_1 f hydrogen ion, 0.10_1 f hydrogen ion and 0.1 f chloride, and 0.10_1 f hydrogen ion and 1 f bromide were prepared, and each solution was represented by (H^+), (H^+, Cl^-) and (H^+, Br^-), respectively, in the following description. The ionic strength of each solution was maintained at 2.1 f. The other experimental conditions are appended to Table IV. The experiment was carried out at the temperature ranging from 280°K to 310°K, and the results are illustrated in Table IV.

The temperature dependence of the pure diffusion-controlled current and of the limiting current is illustrated in Fig. 7. In Fig. 7 the variation of the pure diffusion-controlled current is about 1.9%/deg., which corresponds to the theoretically expected value. On the other hand, the

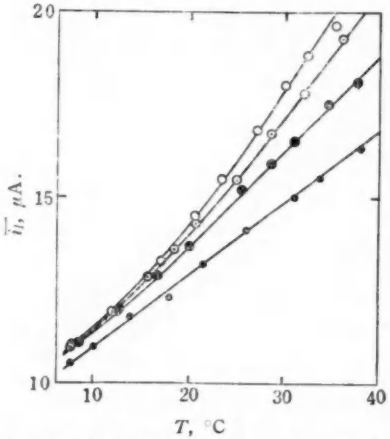


Fig. 7. Temperature dependence of the limiting current. Ionic strength, 2.1 f. (UO_2^{2+}), 4.22 mf. •···pure diffusion-controlled current, ●···0.1 f H^+, ◉···0.1 f H^++0.1 f Cl^-, ○···0.1 f H^++1 f Br^-.

variation in the limiting current is greater than the pure diffusion-controlled current, and it becomes greater in the solution of (H^+), (H^+, Cl^-), (H^+, Br^-) in the order given. As was previously described, the variation in the life-time of the D. M. E. did not appreciably affect the pure diffusion-controlled current. The variation of the reaction time, however, was taken into account and the rate constant was calculated.

In Fig. 8 the logarithm of the rate constant is plotted against the reciprocal of the temperature, and the plot gives a good linear relation in every case. From the tangent of the plot the heat of activation (ΔH^*) was calculated by Arrhenius's

** In this connection the existence of the uranyl nitrate complex can be observed in the catalytic reduction of nitrate at the reduction potential of quinquevalent uranium. For instance, this phenomenon can be observed in Fig. 1 as a drastic increase of the second wave of uranyl in 2 f sodium nitrate solution. Recently, a detailed investigation of this catalytic current was reported by Koryta[14].

14) J. Koryta, *Collection Czechoslov. Chem. Communs.*, **20**, 667 (1955).

equation. The heat of activation (ΔH^*) was calculated by equation 11 in order to apply the absolute rate expression.

$$\Delta H^* = \Delta H^{\ddagger} + RT \tag{11}$$

and the entropy of activation ($\Delta S^{\ddagger}$) was calculated from the equation

$$k = (\epsilon KT/h) \cdot e^{-\Delta H^{\ddagger}/RT} \cdot e^{\Delta S^{\ddagger}/R} \tag{12}$$

where K is the Boltzmann constant and the other symbols have their usual significance. The free energy of activation ($\Delta F^{\ddagger}$) was calculated by the equation

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \tag{13}$$

All these data are illustrated in Table V.

TABLE V
THE HEAT OF ACTIVATION, ΔH^*,‡, THE ENTROPY OF ACTIVATION, $\Delta S^{\ddagger}$, AND THE FREE ENERGY OF ACTIVATION, $\Delta F^{\ddagger}$, OF THE DISPROPORTIONATION REACTION OF U(V)

Medium	ΔH^* kcal./mole	$\Delta H^{\ddagger}$ kcal./mole	$\Delta S^{\ddagger}$ e. u.	$\Delta F^{\ddagger}$ kcal.
2 f $NaClO_4$ +0.1 f H^+	10.8	10.2	−17.1	15.2
1.9 f $NaClO_4$ +0.1f H^+ +0.1f NaCl	13.3	12.7	−2.7	14.1
1f f $NaClO_4$ +0.1f H^+ +1f NaBr	15.4	14.8	+0.4	14.7

(Ionic strength was kept at 2.1 f with $NaClO_4$.)

It seems from the entropy of activation that the specific function is performed by the hydrogen ion in the actual mechanism involving a different character from those of chloride and bromide.

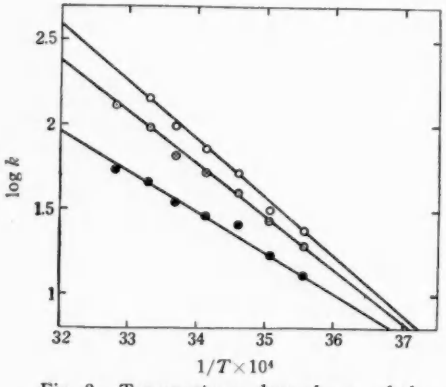


Fig. 8. Temperature dependence of the disproportionation rate constant. Ionic strength, 2.1 f. ●···0.1 f H^+, ⊙···0.1 f H^++0.1 f Cl^-, ○···0.1 f H^++1 f Br^-.

With regard to the function of the hydrogen ion, F. R. Duke and M. C. Pinkerton[15] have proposed a tentative mechanism, that is, the hydrogen ion increases the concentration of $UOOH^{2+}$ (cf. eq. 15) pertaining to the disproportionation and the charge transfer is not performed by the proton transfer but by the hydroxyl-radical transfer. This postulation is supported by the author's result giving a good agreement with an isotopic electron exchange reaction of Fe^{2+}–$FeOH^{2+}$ [4], in which the entropy of activation is −18 e. u., the free energy of activation is 12.2 kcal. and the mechanism of the charge transfer is assumed to be a typical case of the bridge transfer of the hydroxyl-radical. Accordingly, the following mechanism can be confirmed:

$$UO_2^{2+} + \epsilon = UO_2^{+} :$$

Electrode Reaction (14)

$$UO_2^{+} + H^+ \rightleftarrows UOOH^{2+} \tag{15}$$

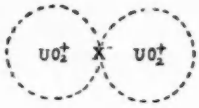
$$UO_2^{+} + UOOH^{2+} = HOUO_2^{+} + OU^{2+} :$$

Disproportionation Reaction (16)

Since we may consider $UOOH^{2+}$ to be a strong acid, the equilibrium in equation 15 shifts toward the right-hand side and results in the increase in the concentration of $UOOH^{2+}$ with the increase in the hydrogen ion concentration. This effect will result in the increase in the disproportionation rate in an acid solution.

On the contrary, an alternate mechanism can be proposed to the cases of chloride and bromide. In Table V it can be seen that the most rapid reaction path has a less negative entropy of activation. Furthermore, in the cases of (H^+, Cl^-) and (H^+, Br^-) the entropies of activation are virtually equal to zero. Since it has been reasoned that the entropy of activation is approximately zero for a unimolecular reaction, the disproportionation is likely to proceed as a unimolecular reaction in these cases. The rate of the disproportionation is of the first order with respect to the anion concentration, and the existence of the uranyl nitrate- or chloride-complex is confirmed, as is previously described. These results lead to the ease of the formation of uranyl complex involving a sharing of the coordination sphere by two center atoms as is formulated in the following:

15) F. R. Duke and M. C. Pinkerton, *J. Am. Chem. Soc.*, **73**, 2361 (1951).



In this state the structure of the two complexes is symmetrical with respect to the shared anion, hence, one can predict that the exchange rate will increase as is clearly enunciated by Libby[16] by applying a quantum mechanical expression of the exchange frequency of the hydrogen molecular ion. It is assumed that the difference in the rate constant among various anions will depend upon the firmness of the coordination, and upon the interatomic distance.

The author wishes to express his hearty gratitude to Professor Teiji Ichikawa, Professor Takeshi Taketa, Professor Mutsuaki Shinagawa and Dr. Hitoshi Yamamura for their advice and encouragement throughout this study.

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16) W. F. Libby, *J. Phys. Chem.*, **56**, 863 (1952).

Analytical Studies of Boron. VI. Determination of Boron Using Ion Exchange Resin

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Introduction

When boric acid was treated with a cation exchange resin, it could be separated quantitatively from cations which coexisted with boric acid. Such a separation procedure was reported by the author[1] and applied to the determination of boron in steels[2], silicon[3] and silicate rocks[4]. For the determination of boron, a sample solution was passed through a cation exchange resin column and the effluent was titrated with an alkaline solution. However, weak acids such as phosphoric and silicic interfere with the titration.

After a solution containing a small amount of boron was treated with an ion exchange resin column, a colorimetric procedure was applied to the effluent. The results were satisfactory.

Treatment of Boric Acid Solution with an Ion Exchange Resin Column

Reagents and Chemicals: *Resins*; Cation exchange resins, Amberlite IR-120 and Diaion K in Na- or H-form.

Anion exchange resins, Amberlite IRA-400 and Diaion A in Cl- or OH-form.

Washing solution; Three normal aqueous solution of chemically pure grade sodium hydroxide, hydrochloric acid and sodium chloride.

Sodium carbonate; Guaranteed grade sodium carbonate.

pH regulating solution; One normal guaranteed grade sodium hydroxide. One normal guaranteed grade hydrochloric acid.

Standard boric acid solution; Guaranteed grade boric acid was recystallized and a solution containing 1.1mg. B/cc. was prepared. This solution was diluted to the given concentration before use.

Preliminary Experiment: Standard boric acid solutions containing 1 γ—20 mg. B were taken, and made up to 20 cc. with distilled water. These solutions were divided into 3 groups. The first group, (a), contained 1—20 mg. B, the second, (b), 10—500 γB, and the third, (c), 1—9 γB.

One normal hydrochloric acid and 1 N sodium hydroxide solution were added to 20 cc. of the boric acid solution and its pH was adjusted to various values. The resulting solution was passed through a cation exchange resin column. The column was washed with distilled water, and a titration method was applied to the (a) group. To the (b) group containing 10—500 γB was applied the colorimetric procedure using acetone-curcumin. The effluent and the washing solution were combined together and diluted to 100 cc. The resulting solution was made alkaline with solid sodium carbonate, evaporated on a water bath,

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1) S. Muto, *J. Chem. Soc. Japan, Pur. Chem. Sec.*, **72**, 976 (1951).

2) J. R. Martin and J. R. Hays, *Anal. Chem.*, **24**, 182 (1952).

3) C. L. Luke, ibid., **27**, 1150 (1955).

4) H. Kramer, ibid., **27**, 144 (1955).

and then the colorimetric procedure was applied. The same procedure was applied to the (c) group. The results are shown in Table I, II and III.

TABLE I

DETERMINATION OF BORON IN (a) GROUP. (mg. B)

Taken	0.0	1.1	5.5	11.	22.
Found	0.01	1.14	5.50	10.9	22.1
	−0.02	1.14	5.47	10.9	22.2
	0.02	1.08	5.51	11.0	21.7
Mean	0.00	1.12	5.49	10.9	22.1
	±0.01	±0.01	±0.01	±0.0	±0.3

TABLE II

DETERMINATION OF BORON IN (b) GROUP. (γB)

Taken	11.	55.	110.	220.	550.
Found	11.3	54.6	110.	215.	554.
	10.1	54.6	107.	281.	546.
	11.4	55.2	105.	221.	546.
Mean	10.9	54.8	107.	218.	548.
	±0.3	±0.2	±1.	±1.	±2.

TABLE III

DETERMINATION OF BORON IN (c) GROUP. (γB)

Taken	0.0	1.1	2.2	5.5	9.9
Found	0.04	1.07	2.24	5.45	9.90
	−0.02	1.05	2.15	5.50	9.84
	−0.02	1.14	2.15	5.48	9.92
Mean	0.00	1.09	2.18	5.48	9.89
	±0.01	±0.02	±0.02	±0.01	±0.02

These results indicate that boron is not adsorbed on the cation exchange resin and separated from cations quantitatively. For the second place, the pH of the boric acid solution, whose volume was diluted to 20 cc., was adjusted by adding hydrochloric acid and sodium hydroxide solutions, for the resulting solution to be passed through an anion exchange resin column of Cl- or OH- form. The direct titration method was applied to the effluent. The content of boron in the effluent is shown in Fig. 1.

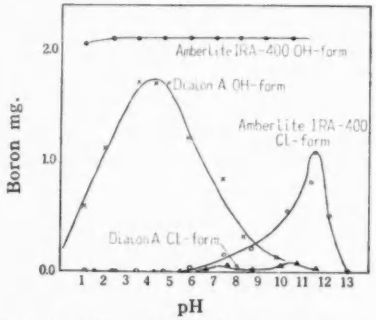

Fig. 1. Boron adsorbed by various forms of anion exchange resins. (The original solution contained 2.12 mg. B.)

Boron was adsorbed to some extent on a weak-base resin, like Diaion A. The adsorption on the resin of OH-form was most marked at pH 3 to 4. The resin of Cl-form did not adsorb boron from acidic solution. When the pH was higher than 5, however, the resin adsorbed boron slightly. A strong-base resin, Amberlite IRA-400, of Cl-form showed the highest adsorption value at pH 11 to 12. When the pH value is higher than 12, the adsorption decreased. Boron was not adsorbed on the resin and separated quatitatively when the pH of solution was lower than 4. This fact makes it possible to separate boron from other anions. When the pH value was higher than 2, Amberlite IRA-400 of OH-form adsorbed boron quantitatively.

A solution containing 5γ boron was prepared from the standard boric acid solution. The solution was treated with the resin column, and the boron in the effluent was determined colorimetrically by the acetone-curcumin method. The results are shown in Fig. 2. The curve has a similar tendency to that in Fig. 1. Boron can be adsorbed to some extent by the Amberlite IRA-400 of Cl-form in the pH range of 11 to 12. It flows down quantitatively when the pH is lower than 5.

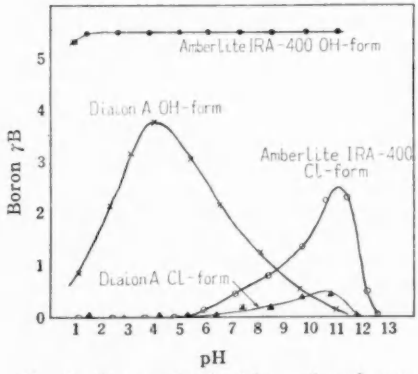

Fig. 2. Boron adsorbed by various forms of anion exchange resins. (The original solution contained 5.5 γB.)

Experimental

Decomposition of Samples: Since the samples are treated with resin column, acid decomposition is better than alkaline fusion. When the samples are decomposed with acid and heated, however, a considerable amount of boric acid volatilizes with water vapor. For this reason, the author placed the sample in a 100 cc. round flask, a slight excess of 6 N hydrochloric acid was added, and the sample was decomposed on

a water bath with a reflux condenser. Samples which could not be decomposed by acids were fused with sodium carbonate of guaranteed grade. The melts were dissolved in a slight excess of 6 N hydrochloric acid.

In order to examine this decomposition method, the guaranteed grade sodium carbonate was added to the standard boric acid solution to make the

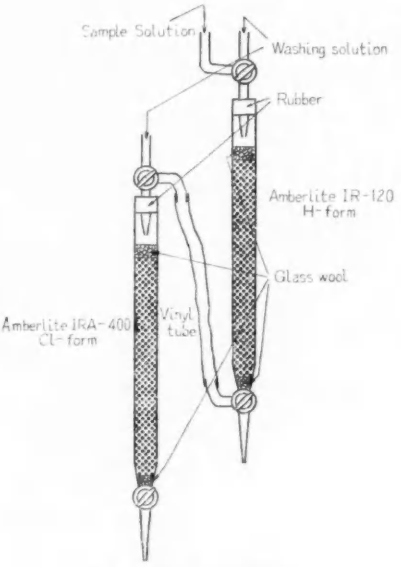

Fig. 3. Resin columns.

solution alkaline, and evaporated on a water bath. The residue was dissolved with a small amount of water and the solution was poured into a 100 cc. round flask. This solution was neutralized with 6 N hydrochloric acid and 1 cc. excess of the acid added. The flask was connected to a reflux condenser and heated on a water bath for 1 hour. The solution was diluted with 50—100 cc. of water and passed through the cation and the anion exchange resin column using the apparatus as shown in Fig. 3. The effluent was analyzed by either titrimetric or colorimetric procedure.

Titrimetric Procedure:

Reagents and Chemicals: Standard sodium hydroxide solution (N/20); Guaranteed grade sodium hydroxide was dissolved in carbonate-free distilled water. The factor of the solution was 1.165.

Sodium hydroxide solution (1 N); Guaranteed grade sodium hydroxide was dissolved in carbonate-free distilled water.

Hydrochloric acid (1 N); Distilled hydrochloric acid was diluted to 1 N.

p-Nitrophenol indicator; 0.1% ethanolic solution.

Phenolphthalein indicator; 0.1% ethanolic solution.

Mannitol; Analytical grade reagent in solid.

Procedure: After a sample solution was treated with a resin column, 1—5 mg. B was pipetted out and poured into a 300 cc. conical flask and the volume of the solution was adjusted to 100 cc. To this solution was applied an improved Foote's titrimetric procedure[5]. One drop of *p*-nitrophenol solution was added as indicator and the solution was neutralized with 1 N sodium hydroxide solution until yellow color of the indicator just appeared. One drop of 1 N hydrochloric acid was added to this solution to make the yellow color vanish, then the flask was connected to an aspirator. The solution was heated and boiled at a low pressure. Carbon dioxide gas being bubbled out, the pH of the solution became higher. When the yellow color of the indicator appeared again, one drop of 1 N hydrochloric acid was added and then the boiling was continued. After 2 to 3 minutes of boiling, the aspirator was removed and the solution was cooled by cold water. One twentieth normal sodium hydroxide solution was added to the solution until the yellow color of *p*-nitrophenol was just visible. This is the starting point of the titration. Two grams of mannitol and 5 drops of phenolphthalein were added to the solution, which was titrated with N/20 sodium hydroxide solution. At the end point, the color of the solution was yellowish brown. The color of phenolphthalein appears at pH 8.0, and the neutralization point of mannitol-boric acid complex lies at pH 7.6. Therefore, the end point of the titration is shown by the appearance of yellowish brown color in the solution.

TABLE IV

DETERMINATION OF BORON BY TITRIMETRIC PROCEDURE. (mg. B)

Taken	0.0	1.1	5.5	11.	22.
Found	−0.04	1.05	5.50	10.7	21.6
	0.03	1.09	5.45	11.3	22.0
	−0.03	1.13	5.54	10.9	22.4
Mean	−0.01	1.09	5.50	11.0	22.0
	±0.01	±0.02	±0.02	±0.04	±0.02

When the yellowish brown color changes to yellow or sometimes disappears on the addition of 2 grams of mannitol, the titration should be continued. The results on the experiment with known amounts of boron is shown in Table IV.

Colorimetric Procedure:

Reagents and Chemicals: Sodium carbonate; Guaranteed grade sodium carbonate (solid).

Hydrochloric acid-oxalic acid solution; Mix one volume of distilled 6 N hydrochloric acid with four volumes of saturated solution of guaranteed grade oxalic acid.

Acetone-curcumin solution; Dissolve 0.0625 g. of curcumin in 5 cc. of carbitol (monomethyl ether of diethyl glycol), then dilute to 500 cc. with acetone. The solution is stable for 2 to 3 months.

Acetone; Distilled with calcium oxide.

5) F. J. Foote, *Ind. Eng. Chem., Anal. Ed.*, **4**, 39 (1932).

Procedure: The Naftel's[6] and the Silverman's[7] method were modified and the following procedure was applied to the sample. After the sample solution was treated with resin, a portion containing 1 to 5 γB was pipetted out and poured into a 50 cc. porclain dish, to which 0.2 g. of sodium carbonate was added. The resulting solution was evaporated on a water bath and acidified with 2 cc. of the hydrochloric acid-oxalic acid solution. The product was evaporated on a water bath at 55±2°C. The heating was continued for 30 min. after the solution was dried. The red color of rosocyanine appeared in the presence of boron. After the colored residue was cooled, it was treated with acetone. The colored acetone solution was centrifuged for 20 minutes at 3000 r. p. m. The clear solution was poured into a 10 mm cell of Hitachi APO-I photocolorimeter equipped with No. G (520 mμ) filter. This method was applied to solutions containing a known amount of boric acid, which were not treated with the resin columns. A known amount of the standard boric acid solution was treated by the above mentioned procedure and the scale of photocolorimeter was plotted in Fig. 4. The results are shown in Table V.

TABLE V
DETERMINATION OF BORON BY COLORIMETRIC PROCEDURE. (γB)

Taken	0.0	1.1	2.2	5.5	11.
Found	0.04	1.10	2.18	5.43	10.8
	0.02	1.08	2.18	5.51	10.6
	0.00	1.06	2.18	5.49	11.3
Mean	0.02 ±0.01	1.08 ±0.01	2.18 ±0.00	5.48 ±0.02	10.9 ±0.1

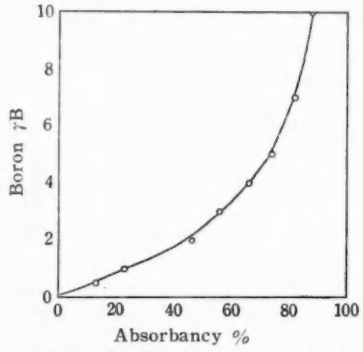

Fig. 4. Standard curve of boron-absorbancy.

This procedure could be applied to a sample containing a small quantity of boron within the error of ±5%.

As shown in Table VI and VII, iron and aluminium interfered with this colorimetric procedure.

6) J. A. Naftel, *Ind. Eng. Chem., Anal. Ed.*, **11**, 407 (1939).
7) L. Silverman and K. Trego, *Anal. Chem.*, **25**, 1264 (1953).

TABLE VI
INTERFERENCE OF IRON. (γ)

B (taken)	Fe^{3+}	B (found)	Error γB
0.0	0	0.02	0.02
0.0	9	0.02	0.02
0.0	45	0.05	0.05
0.0	90	0.05	0.05
0.0	900	0.14	0.14
0.0	9000	0.00	0.00
3.3	0	3.33	0.03
3.3	9	3.40	0.07
3.3	45	3.40	0.07
3.3	90	3.65	0.35
3.3	900	3.85	0.55
3.3	9000	0.08	−2.22
5.5	0	5.45	0.05
5.5	9	5.54	0.04
5.5	45	5.55	0.05
5.5	90	5.63	0.13
5.5	900	6.00	0.45
5.5	9000	0.11	−4.39

TABLE VII
INTERFERENCE OF ALUMINIUM. (γ)

B (taken)	Al	B (found)	Error γB
0.0	0	−0.01	−0.01
0.0	0.5	0.00	0.00
0.0	1	−0.02	−0.02
0.0	10	0.04	0.04
0.0	100	−0.01	−0.01
0.0	1000	−0.02	−0.02
3.3	0	3.32	0.02
3.3	0.5	3.29	−0.01
3.3	1	3.18	−0.12
3.3	10	3.10	−0.20
3.3	100	3.09	−0.21
3.3	1000	3.05	−0.25
5.5	0	5.48	−0.02
5.5	0.5	5.50	0.00
5.5	1	5.36	−0.14
5.5	10	5.24	−0.26
5.5	100	5.24	−0.26
5.5	1000	5.08	−0.42

When the ferric ion exists in small quantities, the error tends to become larger. The presence of the aluminium ion gives negative error. Sodium chloride does not interfere with this method.

Application

Kotoite, paigeite and tourmaline were sampled. Kotoite and paigeite were decomposed with hydrochloric acid in a flask connected with a reflux condenser. Tourmaline was fused with sodium carbonate and the melt was dissolved in hydrochloric acid. When the samples were rich in

TABLE VIII

BORON IN BORON MINERALS

(Determined on the regulated volume of the effluent as explained before)

	Determined		Added		Determined (total)		Recovered	
	Tit.(i) mg. B	Colo.(ii) γB	Tit. mg. B	Colo. γB	Tit.(iii) mg. B	Colo.(iv) γB	(iii)-(i) mg. B	(iv)-(ii) γB
Kotoite	1.77	1.75	1.1	0.11	2.90	1.82	1.3	0.07
(Miyako, Iwate.)	1.72	1.82	5.5	1.1	7.21	2.89	5.4	1.1
5.38±0.06% B_2O_3	1.80	1.73	11.	5.5	12.85	7.17	11.	5.4
Paigeite	5.67	5.55	1.1	0.11	6.82	5.61	1.2	0.06
(Kamaishi, Iwate.)	5.65	5.80	5.5	1.1	11.05	6.86	5.4	1.1
16.00±0.12% B_2O_3	5.67	5.65	11.	5.5	16.80	11.25	11.	5.6
Tourmaline	5.13	5.20	1.1	0.11	6.17	5.28	1.0	0.08
(Shima, Gunma.)	5.22	5.16	5.5	1.1	10.62	6.30	5.4	1.1
7.93±0.04% B_2O_3	5.19	5.14	11.	5.5	16.20	10.57	11.	5.4

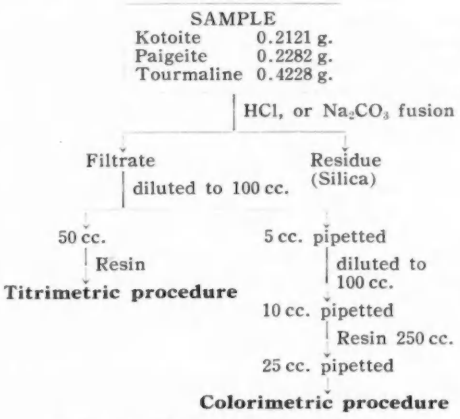

Fig. 5. Decomposition of samples

silica, the acidic solution had to be filtered. The solutions were treated with resin columns, as shown in the Fig. 5. The results in Table VIII were obtained when boron in the effluent was determined by the titrimetric and the colorimetric procedure. The errors of the colorimetric procedure were greater than those of the titrimetric procedure, but less than ±5%. The accuracy of the procedures was examined by adding a known amount of the standard boric acid solution to the minerals. The results were satisfactory as shown in Table VIII. The boron could be determined by the titrimetric procedure, but the colorimetric procedure gave more satisfactory results for micro amounts of boron.

The author wishes to express his sincere thanks to Dr. N. Yamagata and Dr. T. Watanabe for their helpful suggestions and aids during the course of the present investigation.

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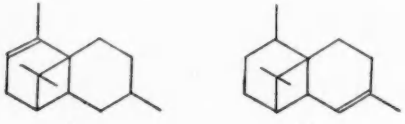
*Polyterpenes I. Permanganate Oxidation of Thujopsene, Part 1**

By Saburo AKIYOSHI and Shizuo NAGAHAMA

(Received June 18, 1957)

Hiba, *Thujopsis dolabrata Sieb. et Zucc.* is an arbor native to Japan, abounding especially in the northern parts of Honshū island, namely Ōu, Hokuriku, and Tōhoku district. In view of the strong resisting power of Hiba wood against saprophytes, the wood oil has long been a target of much attention, and both Yano[1] and Uchida[2] proved in their early investigation that its main constituent is a tricyclic sesquiterpene, which was subsequently named thujopsene by Kawamura[3]. It may be noted that the anti-saprophytic property is due to the minor acidic parts of the oil, the components of which were elucidated by Nozoe, Yasue and Yamane[4].

By the permanganate oxidation of thujopsene, Kawamura[3] obtained a glycol $C_{15}H_{26}O_2$, m.p. 92—3°, a keto-aldehyde $C_{15}H_{24}O_2$, b. p. 170—7°/11 mm, disemicarbazone m. p. 221° (decomp.), and a keto-carboxylic acid $C_{15}H_{24}O_3$, b. p. 180—5°/4 mm, semicarbazone m. p. 219° (decomp.). The ketonic acid was further degradated with hypobromite to a dicarboxylic acid $C_{14}H_{22}O_4$, m. p. 208—9°. On the basis of these degradations and several other observations, he suggested the structure A or B for thujopsene. Unfortunately little has been known about this paper in foreign countries, as it was written only in Japanese and was not introduced in abstracting journals.



A B

Structures A and B suggested by Kawamura.

Later Obata and Fukushi[5] stated that they could not find thujopsene in Hiba wood oil and that its main constituent has the constants between the dicyclic and the tricyclic. To make clear of this discrepancy, Fujita[6] collected ten samples of Hiba wood from various districts where it grows, and confirmed the fact that the main constituent of the oil obtained from them was a tricyclic sesquiterpene. Recently Tanaka and Aikawa[7] reported that thujopsene is a mixture of endo- and semicyclic double bond isomer.

As it is regrettable that the study on the structure of this interesting substance has been interrupted for long years, the authors have decided to take it up at this time. The present paper deals with some of the degradation products by the oxidation of thujopsene with potassium permanganate in acetone solution. Unlike the procedure followed by Kawamura, the authors did not add water to the reaction mixture.

The main product was a crystalline keto-carboxylic acid $C_{15}H_{24}O_3$ (I), m. p. 164—5°, which was oxidized with hypobromite to a dicarboxylic acid $C_{14}H_{22}O_4$, m. p. 210°. This dicarboxylic acid was easily converted into an anhydride m. p. 109—110° by the treatment with acetic anhydride, which shows infrared absorption at 1799 and 1752 cm^{-1} (in carbon tetrachloride), agreeing with glutaric anhydride derivatives[8]. The ring containing double bond, therefore, becomes five membered and Kawamura's formulae are rejected. Since the dicarboxylic acid can be esterified into monomethyl ester m. p. 60°, for the most part, with methanol and sulfuric acid, one of the carboxyl groups may be considered to be attached to the tertiary carbon atom. The ketonic acid I was

* Presented before the 10th Annual Meeting of the Chemical Society of Japan held in Tokyo, April, 1957. Simultaneously two other reports on the same subject were presented independently by Tanaka et al. and Nozoe et al. at this meeting.

1) M. Yano, *J. Soc. Chem. Ind. Japan*, **16**, 443 (1913).
2) S. Uchida, ibid., **31**, 501 (1928).
3) J. Kawamura, *Bull. Govt. Forest Expt. Station*, **30**, 59 (1930).
4) T. Nozoe, A. Yasue and K. Yamane, *Proc. Japan Acad.*, **27**, 15 (1951).
5) Y. Obata and S. Fukushi, *J. Agr. Chem. Soc. Japan*, **26**, 113 (1952).
6) N. Fujita, *Bull. Govt. Forest Expt. Station*, **76**, 175 (1954).
7) J. Tanaka and T. Aikawa, 9th Annual Meeting of the Chemical Society of Japan, Kyoto, April 1956.
8) H. H. Wasserman and H. E. Zimmerman, *J. Am. Chem. Soc.*, **72**, 5787 (1950); G. Stork and R. Breslow, ibid., **75**, 3291 (1953); G. Büchi and R. E. Erickson, ibid., **78**, 1262 (1956).

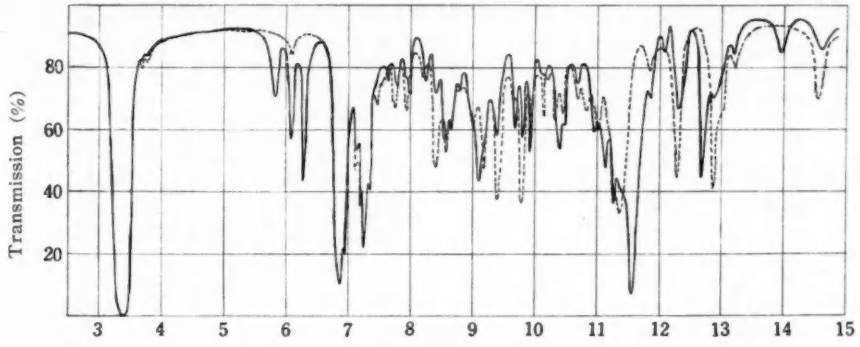


Fig. 1. Infrared spectra of thujopsene and isomerized hydrocarbon.
····· Thujopsene —— Isomerized hydrocarbon

soluble gradually into concentrated hydrobromic acid or sulfuric acid and a ketonic γ-lactone m. p. 56° was obtained from the solution. Curiously, attempted esterification of the acid I with methanol and sulfuric acid gave the same lactone.

Besides this ketonic acid, the authors separated, from the acidic portion of the oxidation product, three crystalline substances, namely an oxylactone $C_{15}H_{22}O_4$ (II), m. p. 123.5°; a hydroxyketonic acid $C_{15}H_{22}O_4$ (III), m. p. 193° (decomp.); and a hydroxylic acid $C_{12}H_{14}O_3$ (IV), m. p. 153°. The acid III was converted into a dehydrated ketonic acid, m. p. 206—7°, by heating it to the decomposing point.

The neutral portion of the oxidation product was fractionated into unchanged thujopsene, isomerized hydrocarbon, and an oxygen-containing high boiling fraction, from which an unsaturated ketone $C_{14}H_{20}O$ (V), m. p. 70° was separated by alumina chromatography.

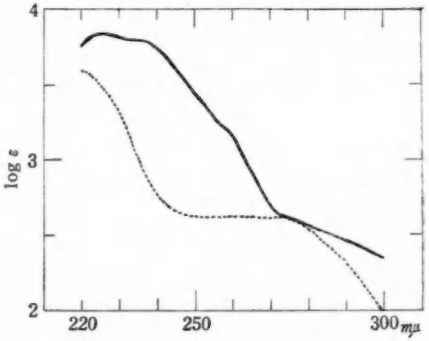


Fig. 2. Ultraviolet spectra of thujopsene (·····) and isomerized hydrocarbon (——) in hexane.

Experimental*

Thujopsene.—Hiba wood oil was purchased from the Soda Aromatic Co. and the Takasago Perfumery Co. The former was transparent, free from acidic portion, d_4^{25} 0.9341, n_D^{21} 1.5035 and the latter raw oil of dark red color, d_4^{15} 0.9518. The thujopsene fraction with following constants was obtained from both oils with an yield of 50—60%. B. p. 121—2°/12 mm, d_4^{25} 0.9311, n_D^{25} 1.5015, $[\alpha]_D^{20}$ −79.7° MR 64.71 infrared and ultraviolet spectra are shown in Figs. 1 and 2.

Permanganate Oxidation.—To a mixture of 100 g. thujopsene and 400 cc. acetone, 120 g. of powdered potassium permanganate was added in portions in the course of 7 hours. The temperature was kept under 30° during the reaction. With the consumption of permanganate, manganese dioxide was filtered off, washed with acetone and the filtrate was concentrated. Manganese dioxide was further extracted several times with hot water, the extract was combined with acetone solution, acidified and extracted with benzene. Then the benzene solution was extracted with 7% sodium bicarbonate solution and 10% sodium hydroxide solution successively, washed with water and dried with sodium sulfate. The semi-solid substance, which was separated from the sodium bicarbonate extract by acidification with hydrochloric acid, was crystallized from benzene. The keto-acid I was obtained at first; removal of the keto-acid I by condensing the mother liquor several times, gave oxylactone (II). Acidifying the sodium hydroxide extract in the presence of benzen precipitate of III was separated between benzene and water layer. From the benzene extract, acid I and acid IV were separated by fractional crystallization. Analytical data of these substances were shown in Table I. Semicarbazone of I, prisms. m. p. 219° (decomp.), 2,4-Dinitrophenylhydrazone of I, yellow, m. p. 187—9°; III, red, m. p. 250—252° (decomp.).

* All melting points are corrected.

TABLE I

OXIDATION PRODUCTS OF THUJOPSENE

	m. p.	Formula	Analysis Found C	Found H	Calcd. C	Calcd. H	Yield* (g.)
I	164—5°	$C_{15}H_{24}O_3$	71.15	9.36	71.39	9.59	4.4
II	122.5—3.5°	$C_{15}H_{22}O_4$	67.47	8.44	67.64	8.33	0.2
III	193° (decomp.)	$C_{15}H_{22}O_4$	67.24 67.89	8.35 8.49	67.64	8.33	0.7
IV	152.5—3.5°	$C_{12}H_{14}O_3$	69.95	7.16	69.88	6.84	0.3
V	68—70°	$C_{14}H_{20}O$	82.09 82.13	9.92 9.89	82.30	9.87	0.2

* In another run, using 200 g. of permanganate, there was obtained 7.0 g of I and 1.4 g. of III.

The neutral portion was about 60 g. and was fractionated into the following three fractions with a concentric column 20 cm high: (1) recollected thujopsene about 30 g.; (2) isomerized hydrocarbon about 10 g. n_D^{25} 1.5111, d_4^{25} 0.9297, MR 65.74: infrared and ultraviolet spectrum Figs. 1 and 2; (3) oxygen-containing high boiling fraction 9 g.

Fraction 3 was chromatographed with alumina column, 5 cm. in diameter and 8 cm. high, developed successively with petroleum ether, benzene, ethyl acetate and methanol. From the benzene eluate a ketone $C_{14}H_{20}O$ (V) was crystallized. It has infrared absorption at 1653 and 1618 cm^{-1} (in Nujol) and is probably an α,β-unsaturated ketone. Semicarbazone m. p. 239° (decomp.).

Dicarboxylic Acid.—The keto-carboxylic acid (I) (2.0 g.) in 10 cc. of 10% sodium hydroxide solution, was added to the hypobromite solution prepared from 8.0 g. sodium hydroxide, 125 cc. water and 3.5 cc. bromine, under ice cooling. After being set aside for 1 hour, the solution was acidified with hydrochloric acid and the needles of dicarboxylic acid were separated. m. p. 205—6° (from dil. ethanol), yield 1.47 g. (73%). The melting point rose to 209.5—210.5° by further recrystallization.

Anal. Found: C, 66.12; H, 8.62; N. E. 127. Calcd. for $C_{14}H_{22}O_4$: C, 66.11; H, 8.72%, N.E. 127.

Dicarboxylic Acid Anhydride.—The dicarboxylic acid (75 mg.) was dissolved in 3 cc. of acetic anhydride and refluxed for 3 hrs. Acetic anhydride was removed under reduced pressure and the residue was crystallized by rubbing. M. p. 109—110° (from acetic anhydride). The anhydride (20 mg.) was added to 0.4 cc. of 10% sodium hydroxide solution, allowed to stand overnight, warmed for few min., and then acidified. The crystals obtained were recrystallized from dil. ethanol, m. p. 210.5—211°. This was mixed with dicarboxylic acid (m. p. 208°) and melted at 208—210°. The infrared spectra of the two coincided.

Esters of Dicarboxylic Acid.—A mixture of the dicarboxylic acid (494 mg.), methanol (20 cc.), and conc. sulfuric acid (8 drops) was refluxed for 5 hr., poured into ice-water and extracted with ether. The ether solution was extracted twice with 7% sodium bicarbonate solution, washed with water and dried. Evaporation of the ether gave 70 mg. of dimethyl ester m. p. 71—3°. By acidifying the sodium bicarbonate extract with hydrochloric acid, monomethyl ester was obtained in needles, (300 mg.) m. p. 59—60° (from methanol-water). Infrared absorption max. 3490, 1705 cm^{-1} (in Nujol) N. E. 308, calcd. for $C_{15}H_{24}O_4 \cdot 2H_2O$ N. E. 304. The sample was kept in a desiccator for 2 weeks and analysed (infrared max. of 3490 cm^{-1} disappeared, and an ester band appeared at 1736 cm^{-1}).

Anal. Found: C, 67.16; H, 9.27. Calcd. for $C_{15}H_{24}O_4$: C, 67.13; H, 9.02%.

Saponification of the monomethyl ester gave the dicarboxylic acid m. p. 209—210°.

Isomerization of the Keto-acid (I) to the Keto-lactone.— (a) To 10 cc. of conc. hydrobromic acid was added 810 mg. of I, which gradually dissolved to yield pink color. After being set aside overnight, the solution was poured onto crushed ice, the needle crystals were collected (470 mg.) and recrystallized twice from ether-petroleum ether mixture and finally with petroleum ether, m. p. 55—56°, yield 200 mg. The ether extract of the aqueous solution produced 100 mg. of the same crystals m. p. 56°. When 50% sulfuric acid was used instead of hydrobromic acid, the same substance was obtained. (b) A mixture of I (100 mg.), methanol (3 cc.) and conc. sulfuric acid (3 drops) was refluxed for 1 hr., poured into water and extracted with ether. The ether was evaporated, and the residue was crystallized by rubbing, yield 75 mg., recrystallized from petroleum ether m. p. 54.5—55.5°. Infrared max. 1774 and 1723 cm^{-1} (in carbon tetrachloride). Semicarbazone, leaflets m. p. 205° (decomp.), 2,4-dinitrophenylhydrazone, yellow orange leaflets m. p. 162°.

Anal. Found: C, 71.42; H, 9.83. Calcd. for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59%.

Thermal Dehydration of Hydroxyketonic Acid (III) — The acid (210 mg.) was heated to 210° in an oil bath. The crystals melted with bubbles, and dehydration was completed within 2 min. When the reaction tube was pulled up from the bath, the product crystallized immediately, m. p. 206—7° (from benzene), yield

130 mg. 2, 4-Dinitrophenylhydrazone, red prisms m. p. 251—3°.

Anal. Found: C, 72.36; H, 8.01. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12%.

The authors wish to express their gratitude to Professor H. Saikachi and Mr. H. Hoshida of the Pharmaceutical Department of this University for rendering help in the measurements of rotation and the elemental analysis. Thanks are due to Mr. M. Shido of the Service Centre of Elementary Analysis of Organic Compounds of this University for undertaking most of the analysis.

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The Structure of Kainic Acid, the Most Active Component of Digenea simplex Ag. I. The Molecular and Crystal Structure of Zinc Kainate Dihydrate*

By Hideo WATASE and Isamu NITTA

(Received May 21, 1957)

Digenea simplex Agardh, distributed over a wide area from the Mediterranean Sea, through the Red Sea and Indian Ocean, to the South Chinese Sea and the southern sea near Japan, is an important seaweed which has been well-known as an anthelmintic since more than one thousand years ago. From the beginning of this century, numerous senior workers of this country endeavoured to find out the active components of this substance. In 1953, Murakami, Takemoto and their collaborators[1] succeeded in isolating the most active component, an acidic and optically active** compound of a comparatively small molecule $C_{10}H_{15}O_4N$, m.p. 251° (decomp.), with an intense anthelmintic effect equivalent to about ten times that of santonin without by-effects. They named it kainic acid (digenic acid) after "*Kaininso*", the Japanese name of the mother alga.

Many studies based on organic chemistry were attempted to determine the chemical structure of this interesting compound. In connection with these we have carried out the structure determination by a more direct method, X-ray crystal analysis.

As an orthodox X-ray procedure applied to a chemically unknown structure, the research was commenced with the zinc salt containing a heavy atom such as zinc. From its electron-density projections along the two principal crystal axes, the three-dimensional structure of the molecule in the zinc salt was determined thoroughly and quantitatively except for the absolute configuration. This X-ray result coincided with the conclusions[2] deduced from the chemical behavior.

This paper deals with the structure determination of the zinc salt in detail.

Experimental

By adding zinc acetate to an aqueous kainic acid solution, two hydrogens of kainic acid are replaced by a zinc atom, and the crystal of the zinc salt with two waters of crystallization is obtained[3]. Crystalline zinc kainate dihydrate ($C_{10}H_{13}O_4NZn \cdot 2H_2O$) is of a colorless prism elongated along a crystal axis chosen as the *c* axis, having a plane of cleavage perpendicular to another principal axis (*a* axis), and insoluble in water and usual organic solvents. It melts with decomposition at higher than 300°C, but one of

* This paper was presented at the Antumnal Joint Meeting of Chemical Societies of Japan, Tokyo, 1956.

1) S. Murakami, T. Takemoto and Z. Shimizu, *J. Pharm. Soc. Japan*, **73**, 1026 (1953); ibid., **74**, 569 (1954).

** $[\alpha]_D^{29}$: −14.8° (*c*=1, H_2O).

2) M. Miyasaki, ibid., **75**, 695 (1955); Y. Ueno, H. Nawa, J. Ueyanagi, H. Morimoto, R. Nakamori and T. Matsuoka, ibid., **75**, 840 (1955); S. Murakami, T. Takemoto, Z. Tei and K. Daigo, ibid., **75**, 869 (1955); H. Morimoto and R. Nakamori, ibid., **76**, 294 (1956); *Proc. Japan Acad.*, **32**, 41 (1956); T. Takemoto, Z. Tei and K. Daigo, *J. Pharm. Soc. Japan*, **76**, 298 (1956).

3) Y. Ueno, H. Nawa, J. Ueyanagi, H. Morimoto, R. Nakamori and T. Matsuoka, ibid., **75**, 807 (1955).

the two waters of crystallization is easily desiccated in a desiccator or by warming it about 40°C, the crystal being broken in pieces to become white powder.

Resulting from the preliminary X-ray examinations, the crystal belonged to the orthorhombic system, and the dimensions of the unit cell were as follows;

$$a = 25.50 \pm 0.07 \text{Å}$$
$$b = 6.93 \pm 0.03 \text{Å}$$
$$c = 7.60 \pm 0.03 \text{Å}$$

Systematic absences of reflexions were recognized only in $(h\ 0\ 0)$, $(0\ k\ 0)$ and $(0\ 0\ l)$ when h, k and l were odd respectively, thus leading to the space group of D_2^4-$P2_12_12_1$. Since the unit cell with this crystal symmetry has four crystallographic equivalent general positions[4], it must contain four molecules or integral multiple of four. Judging from the volume of the unit cell, in the present case, four chemical formula units were contained in the unit cell, namely an asymmetric unit was one formula unit.

The axial periods of the b and c axes seemed to be short enough to permit a good resolution of many of the atoms, although the a axis was too long for a projection to provide any useful picture of the structure, and so the investigation was directed to determination of the projections along the b and c axes.

Two specimens of the single crystal were prepared to take the photographs around the b and c axes. The one used for $(h\ 0\ l)$ zone was obtained by cutting the crystal at right angle to the elongated c axis, and the cross-section perpendicular to the b axis was 0.08×0.3 mm. The other used for $(h\ k\ 0)$ zone had a rhombic cross-section with the following diagonals at right angles to the axis of rotation; 0.15 mm. along the a axis and 0.08 mm. along the b axis.

Then, integrated Weissenberg photographs were taken with filtered Cu K_α radiation, rotating each crystal around each axis in limited angular range, and the reflexions from 221 planes of $(h\ 0\ l)$ and 199 of $(h\ k\ 0)$ were observed out of 249 and 227 possible ones respectively. By comparing with a calibrated scale, the intensities of the reflexions were estimated visually, multiple-film technique being used for accurate measurements of the strong and the weak reflexions, of which the relative intensities ranged from 10.000 to 1 for $(h\ 0\ l)$ zone and from 60.000 to 1 for $(h\ k\ 0)$ zone. The corrections for absorption were made for $(h\ 0\ l)$ in the usual way and were neglected for $(h\ k\ 0)$, the linear absorption coefficient for Cu K_α radiation being $\mu = 27.2\ \text{cm}^{-1}$. Relative structure factors were then deduced from these intensity data by the ordinary process.

Heavy-Atom and Trial Methods

The space group involved is D_2^4-$P2_12_12_1$, and there is no center of symmetry in the crystal. For the sake of convenience in practice, each two-dimensional projection was treated separately with the origin lying on the two-fold screw axis so that the origin was a center of symmetry in the projection. Therefore, the origins used in this analysis are not identical in the two different projections. The relation between the coordinates of the two projections along the b and c axes are clearly shown in Fig. 1. In this account, however, the atomic parameters (Table I) and the final electron-density projections (Figs. 2 and 3) are described reducing to the usual cell.

From the general feature of the reflexions of $(h\ 0\ l)$ and $(h\ k\ 0)$, the position of the zinc atom was easily determined without ambiguity, and the contributions of this atom to the structure factors were then calculated from its parameters, assuming a value of $B = 2.0\ \text{Å}^2$ as the temperature factor. By considering the values of the contributions of zinc, the observed structure factors could be nearly reduced from the relative values to those in the absolute scale.

Using the structure factors whose signs appeared to be reliable from the contributions of the zinc atom, the first Fourier summations of the electron-density projections along the b and c axes were calculated, and the maps (I) and (II) of Fig. 1 were derived. As listed in Tables II and III, 100 terms of $F(h\ 0\ l)$ were used for the map (I) projected on (0 1 0) and 79 terms of $F(h\ k\ 0)$ for the map (II) projected on (0 0 1).

In each projection, the peak of the zinc atom was of course distinct in the expected position, having a reasonable height. However, since the signs for a number of the structure factors could not be given from the position of the zinc atom alone, these maps were sufficient neither for giving any useful information about the structure of the molecule nor for a perfectly straightforward analysis. In spite of such difficulties, it still seemed that the projection along the b axis at the final stage would reveal the molecular structure better than the other, and the further analysis was tried with this projection.

Thus, at first, the b axis projection was attacked by the Fourier method, based on the trial-and-error, in reference to the various peaks of the map (I). However, immediately it was judged to be scarcely possible to come to the correct result in

4) "Internationale Tabellen zur Bestimmung von Kristallstrukturen", Bornträger, Berlin (1935); "International Tables for X-ray Crystallography", Kynoch Press, Birmingham (1952).

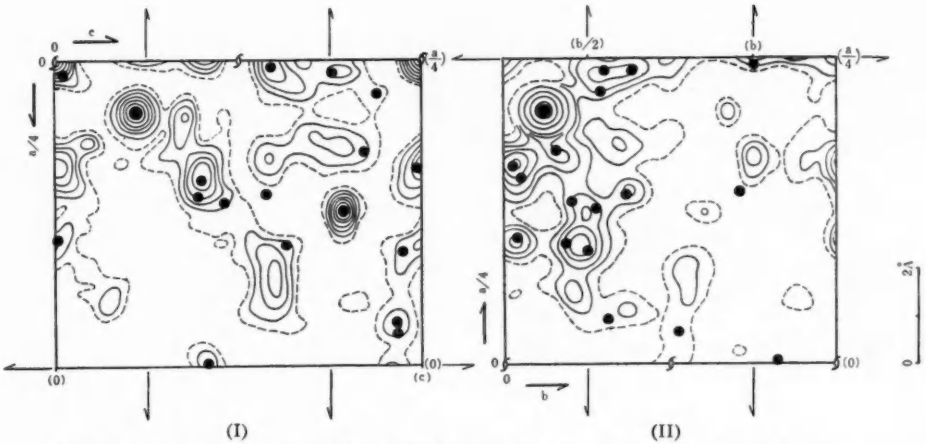


Fig. 1. First Fourier summations of electron-density projections, (I) projected on (0 1 0), (II) projected on (0 0 1). The signs of the structure factors used in the calculations were determined only by zinc. Contours are drawn at equal intervals on an arbitrary scale, but those on the zinc are reduced by a factor of five. Black circles indicate the final atomic positions, that of the zinc being large. The coordinates are taken among those used for the analysis, showing the relation between the two projections, and those to the usual cell are given in brackets.

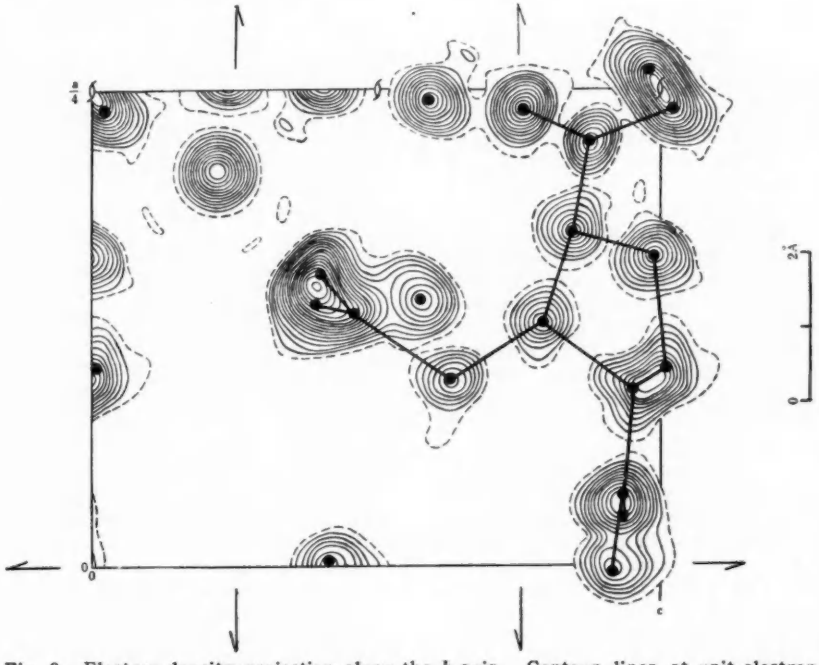


Fig. 2. Electron-density projection along the *b* axis. Contour lines at unit electron intervals, except for those on the zinc at five electrons intervals, the lowest line, broken line, being 3 electrons per Å^2. Black circles show the atomic positions, but the position of the zinc is omitted.

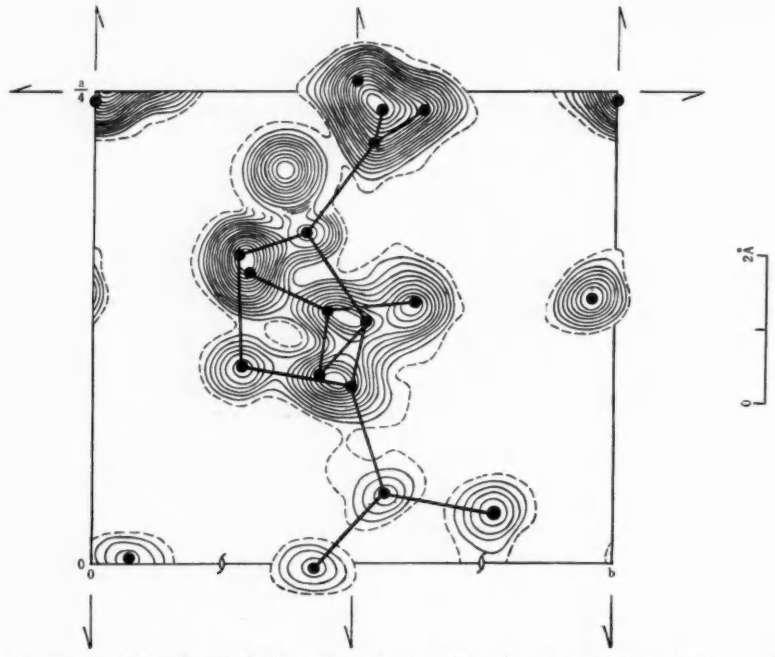


Fig. 3. Electron-density projection along the c axis. Contours and symbols are the same as those of Fig. 2.

such a way, although some of the peaks of this map seemed likely to be real, for example, the peak at the origin, because, in the cases of $h+l$ even, the structure factors with the positive contribution of the zinc were generally greater than its own contribution and those with the negative smaller. In addition to the already fixed zinc atom, these seemingly reliable peaks were made use of as the starting-point, and the structure factors of the lower order were tried without exception quantitatively. Thus an approximate distribution of the atoms in this projection being made reliable for the lower orders, the trial was carried on step by step from the lower to the higher orders, until all the atoms were located satisfactorily to permit the Fourier refinements. In this trial, the structure factors with small resultant value but with great contribution of the zinc atom could be used most effectively, and the change in the sign of (8 0 0) was a key to the further analysis. The electron-density projection of Fig. 2 was the final one thus obtained, and the atomic positions were further refined from this map.

In the same way, the two-dimensional analysis along the c axis was examined on the basis of the x parameters determined above. The electron-density projection was derived as shown in Fig. 3 and the atomic parameters of this projection were obtained. The x parameters for the two projections were in complete

TABLE I
ATOMIC PARAMETERS

Atom	x/a	y/b	z/c
Zn	0.2081	0.3643	0.220
N	0.162_5	0.273	0.987
C_1	0.222_3	0.533	0.873
C_2	0.175_0	0.407	0.840
C_3	0.127_5	0.523	0.790
C_4	0.093_4	0.497	0.950
C_5	0.104_1	0.287	0.007
C_6	0.099_2	0.433	0.627
C_7	0.133_3	0.450	0.460
C_8	0.036_7	0.560	0.933
C_9	0.025_8	0.773	0.933
C_{10}	0.002_5	0.073	0.413
O_1	0.240_0	0.550	0.020
O_2	0.240_9	0.630	0.757
O_3	0.152_5	0.300	0.400
O_4	0.138_3	0.617	0.390
$O_5(H_2O)$	0.245_0	0.000	0.590
$O_6(H_2O)$	0.140_8	0.957	0.573

agreement. All the parameters are listed in Table I (to the usual cell).

At this stage, the discrepancy indices ($R=\sum||F_o|-|F_c||/\sum|F_o|$) are 0.156 for ($h$ 0 l) and 0.146 for (h k 0), employing the data up to sin $\theta/\lambda=0.640$, assuming the values of the unobserved structure factors to be precisely zero, and neglecting the contribution of the hydrogen atoms. In the structure-factor calculations, the values $B=2.0$ Å^2 for (h 0 l), with the exception of (h 0 0), and 3.0 Å^2 for (h k 0) and (h 0 0) were used as the temperature factors common to all the atoms. The atomic scattering factors were taken for zinc from the Internationale Tabellen[4] modifying as Zn^{++} and for the other three from McWeeny's paper[5]; for oxygen $f_o=1/3(f_{\parallel}+2f_{\perp})$ and for carbon the value for 'valence states'.

The observed and the calculated structure factors together with the contributions of the zinc atom are listed in Tables II and III.

TABLE II

OBSERVED AND CALCULATED STRUCTURE FACTORS $F(h\ 0\ l)$, TOGETHER WITH THE CONTRIBUTIONS OF THE ZINC ATOM

The values for (h 0 0) are listed in Table III.

* These structure factors were employed on the first Fourier summations (Fig. 1), using the signs of F_{Zn}.

h k l	$1/4F_o$	$1/4F_c$	$1/4F_{Zn}$
1 0 1	6.6	6.7	4.8
2 0 1	1.2	− 0.8	−12.5
3 0 1	5.8	6.3	3.4
4 0 1	22.8*	−22.0	−21.7
5 0 1	21.5	24.6	1.2
6 0 1	15.0*	−15.2	−23.3
7 0 1	8.1	− 8.2	− 1.1
8 0 1	10.9*	− 8.9	−18.3
9 0 1	15.0	−15.3	− 2.9
10 0 1	4.6	− 4.8	− 9.5
11 0 1	11.1	−10.7	− 3.4
12 0 1	2.7	− 2.6	0.0
13 0 1	12.0	13.1	− 3.1
14 0 1	12.3*	12.9	7.7
15 0 1	2.4	1.9	− 2.0
16 0 1	19.6*	19.8	12.6
17 0 1	10.5	−10.8	− 0.7
18 0 1	16.6*	16.7	12.7
19 0 1	3.3	− 5.1	0.7
20 0 1	13.3*	13.0	9.6
21 0 1	—	− 0.5	1.5
22 0 1	3.2*	3.9	4.8
23 0 1	3.2	5.8	1.7
24 0 1	—	− 0.7	− 0.5
25 0 1	—	0.2	1.5
26 0 1	2.7*	− 3.4	− 4.3
27 0 1	—	− 0.5	1.0
28 0 1	2.5*	− 3.2	− 6.2
29 0 1	—	− 2.5	0.3
30 0 1	4.6*	− 6.0	− 6.1
31 0 1	—	0.3	− 0.3
32 0 1	2.2*	− 3.9	− 4.5
0 0 2	8.5	9.3	−22.1
1 0 2	15.2	17.9	− 2.1
2 0 2	25.6*	−23.9	−19.1
3 0 2	3.0	− 0.1	− 6.3
4 0 2	16.6*	−16.7	−10.2
5 0 2	3.5	3.6	− 8.0
6 0 2	4.0	− 3.8	0.0
7 0 2	22.2*	−20.7	− 7.5
8 0 2	18.0*	18.9	10.2
9 0 2	9.7	− 8.4	− 4.9
10 0 2	19.5*	20.8	15.2
11 0 2	2.2	2.3	− 1.6
12 0 2	22.2*	19.9	15.8
13 0 2	2.5	4.3	1.4
14 0 2	12.7*	12.7	12.3
15 0 2	2.7	2.6	3.9
16 0 2	—	0.6	6.2
17 0 2	2.8	2.3	4.7
18 0 2	—	2.5	0.0
19 0 2	4.5	4.1	4.1
20 0 2	—	− 1.3	− 5.6
21 0 2	—	0.2	2.6
22 0 2	7.4*	− 7.9	− 7.9
23 0 2	3.2	− 3.7	0.8
24 0 2	6.7*	− 8.8	− 8.1
25 0 2	—	− 1.4	− 0.9
26 0 2	4.6*	− 5.5	− 6.1
27 0 2	3.7	− 4.2	− 2.0
28 0 2	1.8	− 1.7	− 3.1
29 0 2	3.0	− 3.3	− 2.3
30 0 2	1.6	− 1.6	0.4
31 0 2	2.8	− 4.2	− 1.9
32 0 2	2.3	3.1	2.6
1 0 3	3.1	− 2.3	−10.7
2 0 3	5.8	7.6	8.4
3 0 3	12.6	−13.0	− 7.5
4 0 3	5.0	5.1	14.6
5 0 3	1.3	1.8	− 2.5
6 0 3	26.2*	26.0	16.0
7 0 3	10.4	8.1	2.4
8 0 3	17.8*	15.2	12.6
9 0 3	8.4*	9.7	6.7
10 0 3	4.1	5.1	6.8
11 0 3	13.4*	11.7	8.2
12 0 3	7.8	− 8.1	0.0
13 0 3	17.2*	15.7	7.5
14 0 3	3.1	− 2.6	− 5.7
15 0 3	3.5	3.5	4.8
16 0 3	9.2*	− 7.8	− 9.3

5) R. McWeeny, *Acta Cryst.*, **4**, 513 (1951).

17 0 3	3.0	− 2.2	1.5	15 0 5	3.9*	− 4.9	− 5.6
18 0 3	15.3*	−13.9	− 9.5	16 0 5	3.6*	3.2	5.0
19 0 3	3.4	3.0	− 1.8	17 0 5	—	− 0.1	− 1.9
20 0 3	7.5*	− 7.0	− 7.2	18 0 5	3.2*	3.9	5.2
21 0 3	—	1.5	− 3.7	19 0 5	3.8	5.4	2.1
22 0 3	5.1*	− 6.0	− 3.7	20 0 5	5.1*	7.1	4.1
23 0 3	3.5*	− 4.1	− 4.5	21 0 5	7.7*	8.4	4.6
24 0 3	3.1	− 0.6	0.4	22 0 5	2.7	2.7	2.1
25 0 3	3.8*	− 5.5	− 3.9	23 0 5	5.3*	4.7	5.5
26 0 3	1.8	0.2	3.3	24 0 5	2.6	2.2	− 0.3
27 0 3	2.6	− 2.4	− 2.5	25 0 5	6.9*	6.2	4.8
28 0 3	3.9*	5.5	4.7	26 0 5	1.1	− 1.1	− 1.9
29 0 3	1.7	− 2.0	− 0.8	27 0 5	3.1*	4.1	3.0
30 0 3	2.2*	3.7	4.7	28 0 5	1.8	− 1.7	− 2.7
31 0 3	1.0	0.2	0.9	0 0 6	10.4*	−12.4	− 5.0
0 0 4	10.3*	10.0	12.6	1 0 6	—	0.1	− 2.7
1 0 4	9.9	10.8	2.9	2 0 6	4.2	− 3.2	− 4.3
2 0 4	13.6*	14.2	10.9	3 0 6	10.3*	− 9.9	− 7.6
3 0 4	2.5	1.2	8.5	4 0 6	6.3	− 5.7	− 2.4
4 0 4	8.8	10.1	5.8	5 0 6	16.1*	−13.2	− 9.9
5 0 4	13.8*	12.1	10.9	6 0 6	—	0.7	0.0
6 0 4	4.4	7.9	0.0	7 0 6	12.0*	− 9.4	− 9.6
7 0 4	9.1*	6.9	10.4	8 0 6	3.2	− 4.1	2.4
8 0 4	3.6	− 4.9	− 5.9	9 0 6	7.1*	− 5.6	− 6.5
9 0 4	5.8*	5.7	7.0	10 0 6	—	− 1.2	3.7
10 0 4	9.6*	− 8.4	− 9.2	11 0 6	6.6	− 5.1	− 2.3
11 0 4	—	− 0.6	2.3	12 0 6	4.3	4.7	4.1
12 0 4	7.3*	− 7.9	− 9.7	13 0 6	—	− 1.8	2.1
13 0 4	2.8	− 3.2	− 2.2	14 0 6	4.9*	6.1	3.3
14 0 4	3.1*	− 1.8	− 7.9	15 0 6	5.9*	7.3	5.7
15 0 4	4.8*	− 4.8	− 5.8	16 0 6	3.1	0.8	1.7
16 0 4	2.2	− 1.6	− 3.9	17 0 6	6.8*	5.9	7.0
17 0 4	12.6*	−12.7	− 7.0	18 0 6	4.2	− 5.2	0.0
18 0 4	—	− 0.4	0.0	19 0 6	7.2*	7.3	6.3
19 0 4	8.9*	− 9.0	− 6.2	20 0 6	3.1	− 4.2	− 1.6
20 0 4	3.1*	2.6	3.6	21 0 6	5.1*	3.4	4.1
21 0 4	3.7*	− 4.8	− 4.1	22 0 6	2.6	− 1.4	− 2.3
22 0 4	6.1*	8.1	5.2	23 0 6	—	0.2	1.4
23 0 4	2.8	− 2.6	− 1.3	24 0 6	2.4	− 2.5	− 2.4
24 0 4	6.7*	7.3	5.5	25 0 6	1.3	− 0.7	− 1.5
25 0 4	2.7	1.8	1.5	1 0 7	7.0*	− 4.6	− 8.9
26 0 4	3.7*	3.5	4.0	2 0 7	2.1	3.7	1.1
27 0 4	1.8	1.6	3.1	3 0 7	4.2*	− 2.4	− 6.2
28 0 4	1.6	− 1.0	2.0	4 0 7	3.0	2.8	2.0
29 0 4	2.3*	2.7	3.6	5 0 7	4.7	− 4.8	− 2.2
1 0 5	26.6*	26.8	11.1	6 0 7	—	0.2	2.2
2 0 5	2.3	− 1.7	− 4.0	7 0 7	2.1	3.1	2.1
3 0 5	6.0*	5.9	7.4	8 0 7	3.6	− 3.6	1.8
4 0 5	10.2*	−10.2	− 7.2	9 0 7	5.5*	7.2	6.1
5 0 5	3.8	− 4.5	2.8	10 0 7	4.6	− 2.1	1.0
6 0 5	9.4*	− 7.7	− 8.0	11 0 7	12.2*	12.3	7.7
7 0 5	1.8	3.9	− 2.6	12 0 7	2.0	− 1.2	0.0
8 0 5	9.8*	−10.5	− 6.5	13 0 7	8.5*	7.5	7.2
9 0 5	1.9	1.1	− 7.4	14 0 7	1.9	− 1.1	− 0.9
10 0 5	1.9	− 0.7	− 3.4	15 0 7	3.8*	4.6	4.8
11 0 5	3.4*	− 2.1	− 9.2	16 0 7	3.3	− 4.4	− 1.5
12 0 5	2.0	1.1	0.0	17 0 7	1.9	1.1	1.6
13 0 5	5.1*	− 6.1	− 8.6	18 0 7	4.2	− 4.3	− 1.6
14 0 5	3.1	4.6	2.9	19 0 7	3.7	− 3.0	− 1.8

20 0 7	2.7	− 2.8	− 1.2
21 0 7	2.4	− 1.9	− 3.9
22 0 7	2.2	− 2.3	− 0.6
0 0 8	3.4	2.0	0.5
1 0 8	2.8	1.1	2.0
2 0 8	3.4	4.9	0.5
3 0 8	7.0*	6.9	5.6
4 0 8	—	0.0	0.2
5 0 8	7.9*	6.9	7.3
6 0 8	2.7	2.8	0.0
7 0 8	9.0*	8.6	7.2
8 0 8	2.7	0.8	− 0.2
9 0 8	3.3*	2.9	4.8
10 0 8	—	− 0.1	− 0.4
11 0 8	—	0.3	1.7
12 0 8	2.7	2.0	− 0.4
13 0 8	2.7	− 2.5	− 1.6
14 0 8	—	0.0	− 0.4
15 0 8	4.2*	− 3.3	− 4.3
16 0 8	2.3	− 0.8	− 0.2
17 0 8	5.3*	− 5.6	− 5.3
18 0 8	1.8	− 2.8	0.0
1 0 9	5.5*	6.1	6.0
2 0 9	—	0.2	0.3
3 0 9	4.8*	5.7	4.1
4 0 9	1.8	1.9	0.7
5 0 9	—	1.2	1.5
6 0 9	2.4	1.5	0.8
7 0 9	1.7	0.7	− 1.5
8 0 9	2.3	1.8	0.6
9 0 9	3.1*	− 3.8	− 4.0
10 0 9	2.1	− 2.0	0.3
11 0 9	7.3*	− 5.1	− 5.2
12 0 9	—	− 0.4	0.0

TABLE III

OBSERVED AND CALCULATED STRUCTURE FACTORS $F(h\ k\ 0)$, TOGETHER WITH THE CONTRIBUTIONS OF THE ZINC ATOM.

* These structure factors were employed on the first Fourier summations (Fig. 1), using the signs of F_{Zn}.

$h\ k\ l$	$1/4F_o$	$1/4F_c$	$1/4F_{Zn}$
0 0 0		162	28
2 0 0	42.3*	−43.2	−24.2
4 0 0	2.8	2.6	12.6
6 0 0	0.3	− 0.6	0.0
8 0 0	24.3*	26.8	−12.0
10 0 0	2.3	− 5.0	17.6
12 0 0	6.7	− 6.4	−17.7
14 0 0	8.8	10.0	13.0
16 0 0	9.0	− 8.0	− 6.4
18 0 0	6.2	− 6.6	0.0
20 0 0	11.0*	9.9	5.5
22 0 0	5.0*	− 4.6	− 7.6
24 0 0	10.3*	7.5	7.4
26 0 0	9.2*	− 8.1	− 5.2
28 0 0	4.8*	4.5	2.6
30 0 0	3.1	3.3	0.3
32 0 0	0.9	− 1.0	− 2.0
1 1 0	9.5	10.5	5.0
2 1 0	39.1*	−38.8	− 8.2
3 1 0	27.6*	−29.2	−14.3
4 1 0	13.9*	15.3	14.0
5 1 0	13.6*	12.7	18.1
6 1 0	12.2*	−11.9	−14.9
7 1 0	12.5*	−10.1	−16.7
8 1 0	21.5*	19.6	11.5
9 1 0	4.5	3.3	10.8
10 1 0	13.7*	−15.5	− 6.0
11 1 0	12.5	10.6	− 3.4
12 1 0	—	1.6	0.0
13 1 0	—	− 0.1	− 3.0
14 1 0	—	0.4	5.1
15 1 0	4.8	5.0	7.7
16 1 0	2.3	− 1.5	− 7.4
17 1 0	9.9*	− 9.0	− 9.2
18 1 0	7.0*	6.6	7.3
19 1 0	18.4*	17.0	7.8
20 1 0	1.2	− 1.0	− 5.3
21 1 0	6.5*	− 6.2	− 4.9
22 1 0	4.3	3.8	2.6
23 1 0	1.9	1.7	1.5
24 1 0	1.2	− 1.2	0.3
25 1 0	—	1.9	1.6
26 1 0	2.9	− 2.8	− 2.1
27 1 0	2.8*	− 2.2	− 3.2
28 1 0	6.2*	5.2	3.0
29 1 0	3.5*	2.8	3.6
30 1 0	—	− 1.9	− 2.8
31 1 0	1.7	− 1.8	− 2.9
32 1 0	1.0	0.8	2.0
0 2 0	19.2	−23.1	2.9
1 2 0	18.3*	−19.2	−21.8
2 2 0	8.4	8.1	− 2.5
3 2 0	2.0	0.5	15.1
4 2 0	3.9	− 5.3	1.1
5 2 0	11.2	13.4	− 5.3
6 2 0	17.5	17.1	0.0
7 2 0	16.5	−14.5	− 5.0
8 2 0	17.2	−19.2	− 1.3
9 2 0	22.8*	23.6	13.0
10 2 0	6.3	5.6	1.9
11 2 0	17.4*	−16.9	−15.5
12 2 0	0.6	− 0.9	− 2.0
13 2 0	12.2*	12.0	14.1
14 2 0	2.1	1.1	1.4
15 2 0	11.9*	−11.5	− 8.7
16 2 0	8.5	− 7.7	− 0.6
17 2 0	8.5	6.9	2.8
18 2 0	5.7	4.9	0.0
19 2 0	2.6	2.1	3.1
20 2 0	1.9	0.2	0.7
21 2 0	7.2*	− 7.6	− 6.1
22 2 0	7.2	− 7.1	− 0.9
23 2 0	6.6*	6.3	7.0

24 2 0	1.9	− 1.4	0.9	21 4 0	3.0	− 2.6	− 1.2
25 2 0	7.4*	− 5.7	− 5.9	22 4 0	3.2*	3.5	5.1
26 2 0	1.4	2.0	− 0.6	23 4 0	3.9	3.6	1.4
27 2 0	5.6*	5.1	3.6	24 4 0	4.1*	− 4.8	− 5.2
28 2 0	1.0	− 1.2	0.2	25 4 0	0.7	0.7	− 1.2
29 2 0	—	− 0.6	− 0.9	26 4 0	2.9*	3.1	3.7
30 2 0	—	− 0.9	0.1	27 4 0	—	− 1.2	0.7
31 2 0	1.1	− 0.5	− 1.2	28 4 0	3.2*	− 2.8	− 1.8
1 3 0	1.7	1.3	− 2.6	29 4 0	—	− 0.4	− 0.2
2 3 0	11.7	−12.2	− 7.6	1 5 0	2.4	− 1.9	− 2.6
3 3 0	6.9	− 8.0	7.1	2 5 0	1.7	0.2	2.4
4 3 0	22.9*	22.5	13.4	3 5 0	6.3*	5.5	7.4
5 3 0	12.8*	−11.4	− 9.2	4 5 0	4.3*	− 4.3	− 4.1
6 3 0	10.7*	− 9.1	−14.3	5 5 0	14.9*	−12.1	− 9.7
7 3 0	11.7*	11.5	8.7	6 5 0	7.8*	7.5	4.6
8 3 0	3.7	3.4	11.3	7 5 0	12.1*	10.6	9.2
9 3 0	6.0	− 7.4	− 5.6	8 5 0	2.9	− 2.4	− 3.7
10 3 0	2.7	− 4.1	− 6.0	9 5 0	6.4*	− 5.8	− 6.1
11 3 0	9.2	−11.0	1.8	10 5 0	1.9	4.1	2.0
12 3 0	4.6	6.1	0.0	11 5 0	6.0	7.0	2.1
13 3 0	7.2	7.1	1.6	12 5 0	—	− 0.3	0.0
14 3 0	2.6	3.0	5.3	13 5 0	1.9	3.0	1.9
15 3 0	7.6*	− 7.2	− 4.4	14 5 0	3.0	− 2.7	− 1.8
16 3 0	8.8*	− 8.0	− 7.8	15 5 0	5.5*	− 4.4	− 4.9
17 3 0	4.1*	3.8	5.1	16 5 0	1.2	2.3	2.6
18 3 0	9.2*	8.3	7.7	17 5 0	7.3*	7.5	6.0
19 3 0	5.1*	− 5.4	− 4.4	18 5 0	—	0.2	− 2.7
20 3 0	7.7*	− 5.9	− 5.7	19 5 0	2.9*	− 2.6	− 5.2
21 3 0	4.3*	4.9	2.7	20 5 0	1.1	− 1.0	2.0
22 3 0	—	− 0.8	2.9	21 5 0	4.4*	4.6	3.4
23 3 0	3.8	− 3.4	− 0.9	22 5 0	1.0	− 0.9	− 1.0
24 3 0	2.6	2.6	0.3	23 5 0	—	− 0.9	− 1.1
25 3 0	1.7	0.9	− 1.0	24 5 0	—	− 0.3	− 0.2
26 3 0	1.6	− 2.2	− 2.4	25 5 0	0.8	− 1.0	− 1.1
27 3 0	2.5	2.1	1.9	26 5 0	—	0.4	0.9
28 3 0	2.8*	2.9	3.2	27 5 0	—	1.4	2.3
29 3 0	2.2	− 1.8	− 2.2	0 6 0	3.8	− 1.1	− 3.2
30 3 0	5.4*	− 5.5	− 3.1	1 6 0	3.8	3.6	7.8
0 4 0	4.6	− 5.2	−14.4	2 6 0	4.3	5.4	2.7
1 4 0	9.9	− 8.5	− 3.6	3 6 0	11.6*	− 9.7	− 5.5
2 4 0	12.9*	11.6	12.4	4 6 0	1.9	− 2.0	− 1.5
3 4 0	6.8	5.4	2.5	5 6 0	8.4	7.7	1.9
4 4 0	7.8*	− 8.1	− 6.7	6 6 0	2.7	4.3	0.0
5 4 0	5.6	− 5.8	− 0.8	7 6 0	1.9	2.4	1.8
6 4 0	3.3	− 2.0	0.0	8 6 0	—	0.5	1.5
7 4 0	2.4	− 2.3	− 0.8	9 6 0	6.4*	− 8.3	− 5.0
8 4 0	11.0*	9.4	6.7	10 6 0	1.0	− 1.4	− 2.3
9 4 0	1.5	− 2.7	2.2	11 6 0	6.9*	7.7	6.3
10 4 0	11.6*	−11.1	−10.3	12 6 0	3.0	2.4	2.5
11 4 0	1.1	− 2.0	− 2.7	13 6 0	5.0*	− 5.9	− 5.8
12 4 0	12.7*	11.4	10.6	14 6 0	2.6	− 2.3	− 1.9
13 4 0	1.0	0.3	2.5	15 6 0	1.8	1.5	3.8
14 4 0	11.7*	− 9.6	− 8.0	16 6 0	—	0.5	1.1
15 4 0	2.7	− 2.5	− 1.6	17 6 0	—	− 1.6	− 1.3
16 4 0	1.2	1.1	4.1	18 6 0	1.0	1.3	0.0
17 4 0	3.8	3.0	0.5	19 6 0	1.5	0.9	− 1.4
18 4 0	—	− 0.4	0.0	20 6 0	1.5	− 1.5	− 0.9
19 4 0	—	0.0	0.6	21 6 0	1.0	1.3	2.9
20 4 0	3.0*	− 3.2	− 3.6	22 6 0	1.9	2.0	1.3

23 6 0	3.3*	− 4.7	− 3.4
24 6 0	1.2	− 1.4	− 1.3
1 7 0	—	0.7	0.5
2 7 0	1.9	1.1	2.9
3 7 0	1.2	− 2.4	− 1.5
4 7 0	3.7*	− 4.1	− 5.1
5 7 0	2.6	3.2	1.8
6 7 0	5.2*	6.2	5.7
7 7 0	1.4	− 2.6	− 1.7
8 7 0	5.0*	− 5.3	− 4.7
9 7 0	1.4	1.2	1.2
10 7 0	3.8*	3.8	2.6
11 7 0	—	− 0.7	− 0.4
12 7 0	1.0	0.0	0.0
13 7 0	1.0	− 0.1	− 0.4
14 7 0	3.4*	− 3.2	− 2.4
15 7 0	—	0.1	1.0
16 7 0	4.1*	5.7	3.5
17 7 0	0.8	0.0	− 1.2
18 7 0	2.0*	− 2.4	− 3.6
19 7 0	0.6	1.0	1.1
20 7 0	0.6	2.1	2.7
0 8 0	3.7*	3.4	4.0
1 8 0	—	0.0	2.4
2 8 0	3.4*	− 5.1	− 3.5
3 8 0	—	− 1.2	− 1.7
4 8 0	2.1	2.5	1.8
5 8 0	—	0.0	0.6
6 8 0	0.9	− 1.4	0.0
7 8 0	—	0.2	0.6
8 8 0	0.9	− 1.8	− 1.9
9 8 0	1.8	− 1.8	− 1.6
10 8 0	2.1	2.5	3.0
11 8 0	2.3	2.0	2.0
12 8 0	1.7	− 2.4	− 3.1
13 8 0	3.1	− 3.8	− 1.9
14 8 0	1.2	1.8	2.5

Determination of the Molecular Structure

From the agreement between the corresponding peaks in the two projections, the x, y and z parameters of all the atoms could be easily determined without requiring the three-dimensional analysis. The two water oxygens (O_5, O_6) were recognized by the fact that they are isolated from the other atoms in reasonable distances, and from the knowledge about bond lengths and bond angles the atoms of the molecule could be linked to each other in a reasonable way as indicated in the electron-density maps (Figs. 2 and 3). The four oxygen, one nitrogen and ten carbon atoms of the molecule were recognized to be as illustrated in Fig. 4, and these assignments will be discussed below. The bond lengths and the bond angles, calculated by the atomic parameters of Table I, are shown in Fig. 5, and it may be said these lengths will be correct at least to ±0.04Å.

In the upper, the middle and the lower part of the electron-density maps, there are seen three planar trigonal groupings formed by every four atoms in the molecule. The two of these, the upper and the middle grouping, are considered to be carboxyl groups, judging from the heights of their peaks and the short distances from these to the zinc atom (Figs. 6 and 7), and the bond lengths and the bond angles within these grouping; they are C_2—(C_1—O_1, O_2) and C_6—(C_7—O_3, O_4). The C_3—C_6 distance of 1.56 Å being of carbon-carbon

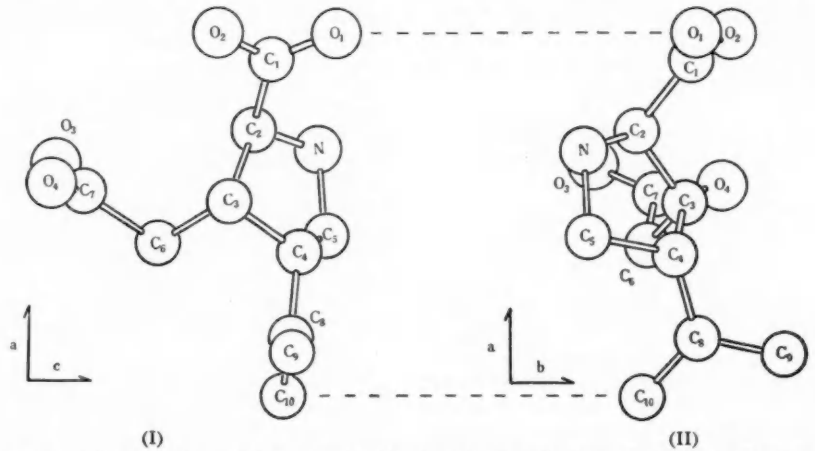


Fig. 4. The structure of kainic acid, (I) viewed along the b axis, (II) viewed along the c axis.

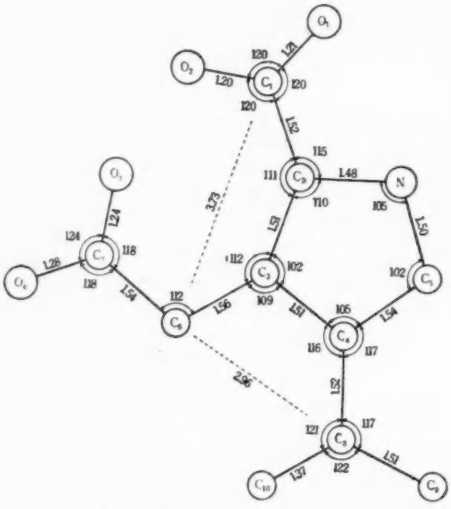


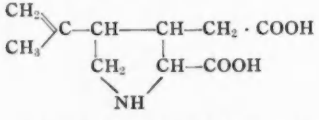
Fig. 5. Bond lengths (Å) and bond angles (°).

single bond, C_3—(C_6—C_7—O_3, O_4) is an acetic acid group.

That of the lower part, having low peaks, is attributable to an isopropenyl group, C_4—(C_8—C_9, C_{10}), since their bond lengths and bond angles are conformable to the characterics of this group, the C_8—C_{10} bond of 1.37 Å being its double bond.

The nitrogen atom is distinguished from the carbon atoms by the short distance from the zinc atom and by the height of its peak higher than the surrounding carbon atoms. The five-membered ring, N, C_2, C_3, C_4, C_5, is of pyrrolidine as identified by the values in Fig. 5.

Thus the structure of kainic acid is concluded to be 2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine.



As for the stereochemical configuration of the molecule, the carboxyl group of C_2 and the acetic acid group of C_3 take the *trans*-configuration relative to the ring, and the latter group and the isopropenyl group of C_4 are in the *cis*-configuration. In the isopropenyl group, the double bond C_8—C_{10} is so located as to be nearer to the ring than the single bond C_8—C_9. These stereochemical configurations are well shown in Fig. 4.

The five-membered pyrrolidine ring is in the present case considerably more distorted from the planar form than that of hydroxyproline[6], and this may owe mainly to the two side-chains attached to the neighboring C_3 and C_4, the side-chains closely approaching each other because of the *cis*-configuration. The distance between C_6 and C_8 is found to be about 3.0 Å, much larger but more adequate than the value of 2.6 Å calculated tentatively using the standard bond distances and bond angles.

Description of the Crystal Structure

The views of the crystal structure along the *b* and *c* axes are illustrated in Figs. 6 and 7, giving the intermolecular distances. The unit cell contains four zinc cations, eight water molecules and four kainate anions, the molecular structure having already given in the last section. As a whole, the crystal structure is a layered one as can be supposed from the plane of cleavage.

The nitrogen, the four oxygens of the two carboxyl groups and the oxygen O_5 of the water surround the zinc, and these six atoms form an octahedron around it. The distances from the zinc to these oxygens, except for Zn...O_4 of 2.81 Å, vary from 2.02 to 2.15 Å, as generally found in the zinc salts of oxygen acids, and the distance between the zinc and the nitrogen is 2.21 Å, again the usual value for Zn...N. While the one water (O_5) is the coordinated water, the other (O_6) does not coordinate to the zinc. It is conceivable that the water O_6 is easily desiccated in a desiccator or by heating.

All the hydrogens of the two waters form the O—H...O hydrogen bonds between the two waters or the waters and oxygens of the carboxyl groups. These hydrogen bond distances are 2.68 Å of O_5—H...O_6, 2.81 Å of O_5—H...O_2, 2.73 Å of O_6—H...O_3 and 2.74 Å of O_6—H...O_4. The hydrogen attached to the nitrogen may be considered also to form the intermolecular hydrogen-bridge between the nitrogen of one molecule and a carboxyl group of another, its N—H...O_1 distance being 2.93 Å.

By all the above-mentioned interactions, the zinc cations, the waters and the kainate anions are linked to each other

6) J. Zussman, *Acta Cryst.*, **4**, 493 (1951); J. Donohue, ibid., **5**, 419 (1952).

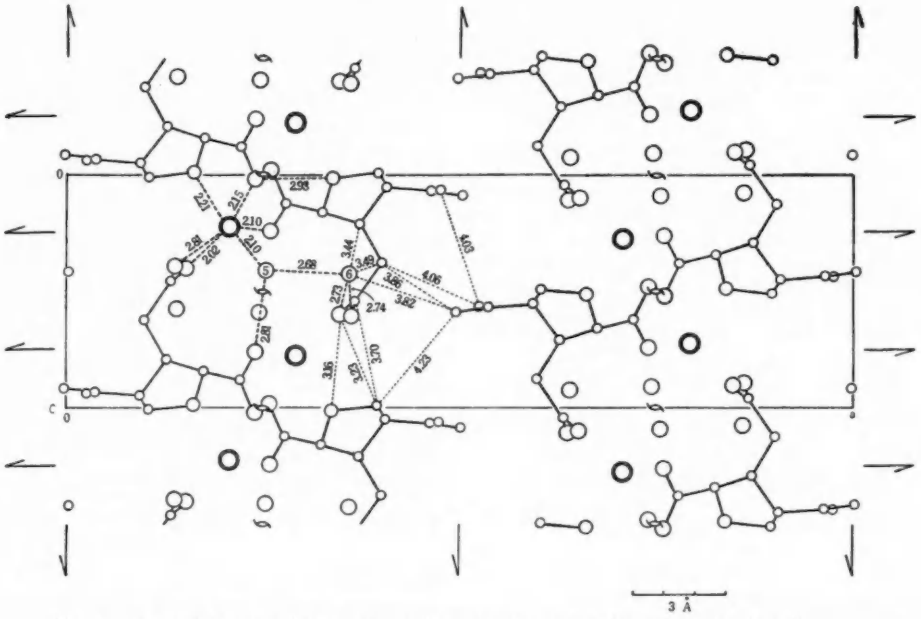


Fig. 6. View of the crystal structure along the *b* axis. The intermolecular distances are shown in Å. Double circles represent zinc, and single circles other atoms, being large, middle and small circles for oxygen, nitrogen and carbon, respectively.

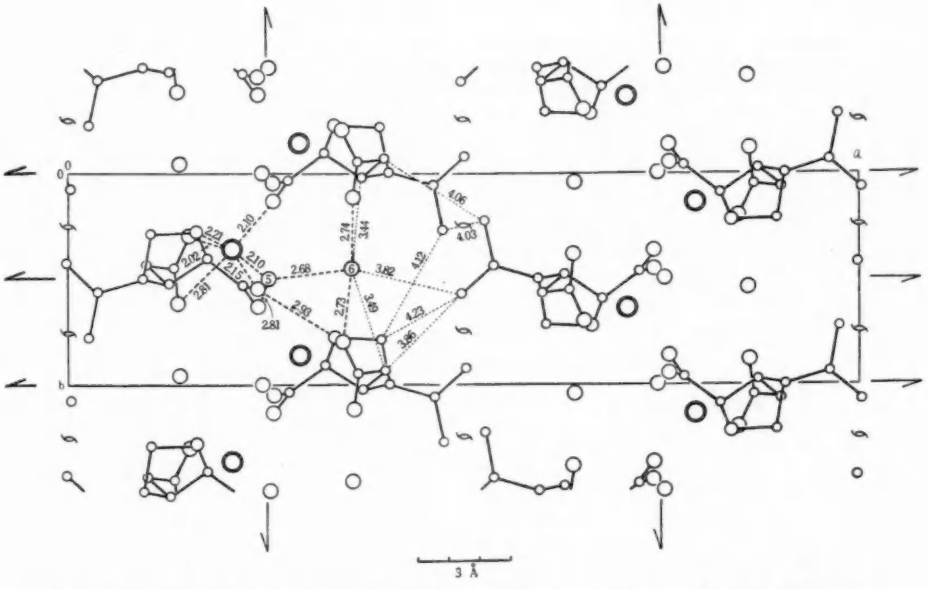


Fig. 7. View of the crystal structure along the *c* axis. The intermolecular distances are shown in Å. Symbols are the same as those of Fig. 6.

forming a layered structure perpendicular to the *a* axis, and between the layers only weak van der Waals attractive forces of the non-polar groups or atoms are operative. The distances of the closest approach between the layers are 3.86 Å of $C_{10}...C_6$, 4.03 Å of $C_9...C_9$ and 4.06 Å of $C_6...C_6$. It is obvious that the cleavage of (1 0 0) plane takes place here.

We wish to express our thanks to Professor-Emeritus S. Murakami, Dr. T. Takemoto and their collaborators of this University, and Drs. S. Kuwada, S. Tatsuoka, Y. Ueno and their collaborators of the Research Laboratories of Takeda Pharmaciutical Industries, Ltd., for supplying the specimens, kind discussions and micro-analyses. Thanks are also due to Drs. Y. Tomiie and H. Tani of this University for helpful advice and continued interest. Appreciations are also expressed to Messrs T. Siba, S. Sakakibara and M. Okumiya and Miss K. Koike of this University for their help in microanalysis.

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Fundamental Studies on the Biogeochemical Prospecting for Manganese

By Noboru YAMAGATA and Toshiko YAMAGATA

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Most of the manganese ores of economic importance in Japan form very small ore bodies near the surface, and the mining industries are generally of very small scale. Systematic prospecting has not yet been performed but only groping has been carried on. Geochemical prospecting for manganese ore by the analyses of soil and surface water has been studied by M. Shima[1] in this country and he suggested such possibilities in several mines. Surface water is not always available; when a representative soil sample is required, the sampling method may become rather complicated. These considerations led the authors to make an attempt of a geochemical prospecting for manganese by use of plant leaves as an aid.

Principle

Several problems are underlying in the application of plant material in practice as an indicator for manganese level in soil. Those problems could be briefly described as follows:

(1) Correlation of manganese level between soil and plants.

Numerous investigators performed experiments on some culture plants by use of water and sand culture method and positive correlation of manganese level between the medium and the plant has been confirmed. This relationship is likely to be applied to natural plants, herbs and trees, although the direct evidence has not been submitted.

Above mentioned correlation is only appropriate for manganese in solution. In our case, the matter in question is the correlation between mineral content of manganese in soil and manganese level in plants. Fortunately, several problems on the soil manganese conditions were solved by G. D. Sherman and P. M. Harmer in 1942[2], and we can use their results as the basis for further research.

(2) In practice, to obtain the plant samples which are as representative as possible, a tree is preferred to small herbaceous plants, because the latter are sensitive to small local variations in soil. Only two grams or less in dry weight is required for the analysis of manganese in plant leaves; two problems arise about the sampling method to avoid variations.

1) M. Shima, "Geochemical Prospecting" (in Japanese) Maruzen Co., Ltd., Tokyo (1955) p. 180.

2) G. D. Sherman and P. M. Harmer, *Soil Sci. Soc. Amer. Proc.*, **7**, 398 (1942).

One of the variations of manganese content is due to the difference in age and position of leaves of a tree, and the other is due to the age of the tree. The dimensions of those variations must be confirmed at the beginning of the experiment.

Experimental

In the chemical analysis of manganese and iron in plant leaves, the air-dried (100°C for 6 hours) samples were first ashed in a porcelain dish over an open flame. The ash was moistened with water, decomposed with 5 ml. of nitric acid (1 : 1) and 2 ml. of sulfuric acid, evaporated to dryness, and then heated to fumes. When the resultant liquid was dark colored, one ml. of hydrogen peroxide solution and 2 ml. of concentrated nitric acid were added and again heated to fumes. To the colorless or pale-yellow liquid was added hot water and insoluble matter was filtered. The filtrate was made up to 50 ml., and a 40 ml. aliquot was used for colorimetric determination of manganese as described in A. O. A. C. official methods of analysis[3], and 10 ml. aliquot for iron determination by use of thiocyanate colorimetric method as usual[4].

Soil extractions were made on freshly taken soil samples, and a separate portion was taken for moisture determination. Further procedure was the same as that of Sherman and Harmer[5].

The manganese mineral content in soil was determined as follows. One to ten grams of soil sample was taken in a beaker and 5 to 30 ml. of concentrated sulfuric acid and 20 to 100 ml. of concentrated nitric acid were added. This was heated gently until brown fumes diminished, and boiled until white fumes appeared. Phosphoric acid was added, boiled for a few minutes and after cooling made to 100 ml. or one litre. A suitable quantity of the clear supernatant solution was taken and the manganese determined as described before. The manganese extraction methods for the various states in soil are schematically shown in Fig. 1.

State of Mn in soil		Extractant
Active-Mn	Water-soluble	H_2O
	Exchangeable	1 N-NH_4-acetate
	Easily-reducible	1 N-NH_4-acetate and hydroquinone
Acid-soluble		$HNO_3+H_2SO_4$

Fig. 1. State of manganese in soil and extractants.

3) "Official Methods of Analysis of the A. O. A. C." 7th ed. Association of Official Agricultural Chemists, Washington (1950).

4) E. B. Sandell, "Colorimetric Determination of Traces of Metals" 2nd Ed., Interscience Publ., Inc., New York (1950).

5) Care must be taken for hydroquinone in use, because commercial hydroquinone frequently contains a considerable amount of manganese.

Errors in the colorimetric analyses of manganese and iron were calculated from the quadruplicate determinations on a powdered and well-mixed plant sample (*Chamaecyparis obtusa* Endlicher, "Hinoki"). The results are shown in Table I. The relative error was 0.4% for manganese and 1.3% for iron.

TABLE I
ERRORS IN THE COLORIMETRIC ANALYSES OF MANGANESE AND IRON IN PLANT MATTER
(On fresh basis)

Chamaecyparis	Mn (p.p.m.)	Fe (p.p.m.)	Mn/Fe
obtusa	78.9	222	0.355
	77.3	216	0.358
	76.9	209	0.368
	77.4	225	0.344
Mean	77.6	218	0.356
Average deviation	0.31	2.8	0.0034

Results and Discussion

Culture Experiments.—At first, a water-culture experiment was carried out with about thirty-days seedlings of tomato plants. The plants had been cultured for two weeks in glazed clay pots with a perfect universal culture solution[6] when the solution was replaced by those of six levels of manganese concentrations. After twenty days culturing with occasional renewal of the solution, the plants were harvested and the leaves were analyzed for manganese.

The result shown in Table II indicates a fair correlation between the manganese content in plants and the manganese level

TABLE II
WATER CULTURE EXPERIMENT WITH *Solanum Lycopersicum* L

Pot No.	Mn level in culture sol. p.p.m.	Mn content in cultured plant p.p.m. (dry basis)	Conc. ratio
1	0.03	74.5	2500
2	0.1	126	1260
3	0.3	196	650
4	1	302	300
5	3	323	100
6	10	1029	100

in culture solution which ranged from 0.03 to 10 p. p. m. But, the concentration ratio of manganese by a plant grows small when the manganese level in solution is raised. In other words, the magnitude of

6) Devised by the authors grounded on the distribution of chemical elements in natural plants; *Japan Analyst*, **6**, 75 (1957).

TABLE III
CULTURE EXPERIMENT ON THE SOIL MIXED WITH RHODONITE
(p. p. m. on dry basis)

Pot No.	pH of soil	Acid soluble Mn	Active Mn			Plants Mn	
			Water soluble Mn	Exchangeable Mn	Easily reducible Mn	Aster sp.	Carex sp.
Contrast	7.1	700	0	7.1	92	95	6.2
1	7.1	710	0	2.4	138	109	13
2	7.0	1400	1.0	11	163	118	17
3	7.0	2350	3.2	23	504	168	33
4	7.0	5440	9.3	52	958	379	71
5	7.0	7000	10	116	1940	622	113
6	7.0	18500	—	—	—	—	3090*

* died in two weeks

TABLE IV
CULTURE EXPERIMENT ON THE SOIL MIXED WITH HAUSMANNITE
(p. p. m. on dry basis)

Pot No.	pH of soil	Acid soluble Mn	Active Mn			Tomato plant	
			Water soluble Mn	Exchangeable Mn	Easily reducible Mn	Mn	Mn/Fe
Contrast	7.2	700	0	7.1	92	174	1.58
1	7.0	1480	2.9	0	239	219	0.90
2	7.2	2130	2.0	1.0	495	203	2.42
3	7.3	4810	3.7	2.1	1700	256	4.57
4	7.3	12900	23	6.5	4520	419	5.66
5	7.3	22800	34	11.5	5740	393	9.82

fluctuations in the manganese content of a plant is smaller than that of manganese in the medium. The former is only 1/24 of the latter in this experiment, and the relationship is also valid in the soil experiments as described later.

Soil culture experiments were carried out with two kinds of manganese mineral mixed with a normal soil. The soil was mixed with various quantities of rhodonite[7] and hausmannite[8] minerals separately and plants were cultured on them for about one month in pots. At the same time when the cultured plants were harvested, fresh soil samples were collected from the pots and extraction of manganese in various states was performed, and then a separate portion of the sample was used for soil reaction test with 100 ml. distilled water by use of glass electrode pH meter. The results are shown in Tables III and IV.

Addition of rhodonite to the soil slightly increased acidity of the soil and vice versa in the case of hausmannite, but the extent of increase or decrease was not remarkable.

When the two soils mixed with almost the same quantity (in acid soluble-manganese) of rhodonite and hausmannite, were compared with respect to their manganese status, the levels of water-soluble and the easily reducible manganese are of comparable order in both soils, but the level of exchangeable manganese in the soil mixed with rhodonite (Table III) is much higher, about twenty times or more, than that in the other soil.

In accordance with the variation of acid-soluble manganese in soil, the manganese content of *Aster* sp. and *Carex* sp. varied from 95 to 622 p. p. m. and from 6.2 to 113 p. p. m. respectively. The magnitude of fluctuation is about 6.5 in the former, and 18 in the latter; in this sense, *Carex* sp. can more sensitively indicate the manganese level in soil than *Aster* sp. but its response for manganese is lower.

In the case of the culture experiment with hausmannite, a tomato plant was used and the magnitude of fluctuation was only about 2 when acid soluble manganese varied from 700 to 22800 p. p. m. In this case iron content of the plant was also determined and Mn/Fe ratio calculated. As shown in Table IV, the fluctuation of Mn/Fe is much greater than that of

7) Contained a small amount of hausmannite and braunite.
8) Contained a small amount of braunite.

manganese content itself. Thus, under certain circumstances, we can employ the ratio of manganese to iron in a plant as the more sensitive indicator for the manganese level in soil.

Variation of Manganese and Iron Content due to the Sampling Position of a Tree.—A "Hinoki" tree (*Chamaecyparis obtusa* ENDLICHER), about thirty years of age, was chosen in the campus of our college. Four samples of leaves, about 200 grams each were pulled off from the south, the east, the north and the west side of the tree, and young leaves were collected from the top of the tree. Those samples were separately analyzed in the same way as described before. The result shown in Table V indicates that the variation due to the sampling position of a tree is great enough to be beyond the errors in determination. Thus, it can be concluded that to obtain leaves which are as representative as possible, a composite sample must be taken from around the tree.

Table V
Variation due to the sampling position of a tree
(*Chamaecyparis obtusa* leaves, on fresh basis)

Sampling position	Mn p.p.m.	Fe p.p.m.	Mn/Fe
East side leaves	25.1	125	0.201
West side leaves	23.2	131	0.177
South side leaves	20.0	121	0.165
North side leaves	29.8	130	0.229
Top young leaves	23.4	82.4	0.284

Variation due to the Age of the Tree.—Five "Hinoki" trees of different ages were chosen in the campus and composite samples were taken for analyses. The result shown in Table VI indicates the increasing manganese content and Mn/Fe ratio of plant in consequence of the growing age. So, it can be concluded that to guess manganese levels in soil we must take foliar samples from the trees of an age as similar as possible, e.g. from twenty to thirty years.

Table VI
Variation due to the age of a tree
(*Chamaecyparis obtusa* leaves, on fresh basis)

Proximate age Years	Mn p.p.m.	Fe p.p.m.	Mn/Fe
3	25.6	265	0.097
10	20.8	155	0.13
20	49.8	169	0.29
30	34.8	133	0.26
40	77.1	212	0.36

Possibility of the Practical Application.—In order to find out whether this method of manganese analysis was applicable to the practical soil investigation and the prospecting of ores and to examine how much the manganese content of a plant varied with circumstances, the authors collected, in September 1956, a number of samples in the mine district near the river Kiriu, about four kilometers from the city of Kiriu.

The bed rock consists of paleozoic chert and slate, and the principal manganese ore consists of rhodochrosite, rhodonite, tephroite, penwithite and various oxide minerals.

Five samples of "Hinoki" (20—30 years) leaves were collected in a non-mineralized district near the river Kiriu about one kilometer away from the mineralized district where eight samples were collected. As shown in Table VII, even in the non-mineralized district, the trees contained much larger quantities, about three times

Table VII
Variation of manganese content of "Hinoki" in mineralized and non-mineralized districts

In mineralized district			In non-mineralized district		
Location No.	Mn p. p. m. dry basis	Mn/Fe	Location No.	Mn p. p. m. dry basis	Mn/Fe
6	569	19.4	1	246	4.6
7	629	13.4	2	169	2.6
8	1228	26.8	3	109	2.1
9	832	29.1	4	196	3.6
10	505	10.1	5	215	4.6
11	817	21.7	Av.	187	3.5
12	485	9.1			
13	656	17.8			
Av.	715	18.4			

TABLE VIII
CORRELATION OF MANGANESE LEVELS BETWEEN SOIL AND PLANT IN THE MINERALIZED DISTRICT

Location No.	pH of soil	Acid soluble Mn p.p.m.	Active Mn			*Quercus acutissima*		*Chamaecyparis obtusa*	
			Water soluble Mn p.p.m.	Exchangeable Mn p.p.m.	Easily red.-Mn p.p.m.	Mn p.p.m.	Mn/Fe	Mn p.p.m.	Mn/Fe
1	7.3	350	0	1.2	16.8	265	4.2	337	5.7
2	7.4	300	0	0.2	19.7	244	3.4	414	9.6
3	7.0	425	0	1.8	71.1	1020	16.4	924	16.4
4*	7.0	1737	0	1.2	424	875	11.6	917	16.8
5*	7.1	5565	0	0.9	720	1050	21.3	671	12.5

* near the deposits.

as much manganese and less iron than those trees in the campus (Table VI).

The Mn/Fe ratio indicates more sensitively those relationships: the ratio in the non-mineralized district is 3.5 on average and this value is more than ten times as great as those in the campus. The ratio grows as big as 29.1 in the mineralized district. The antagonistic behavior of manganese and iron in relation to plant uptake is a very interesting object of physiological research, although it is beyond the scope of this article.

Each of five samples of "Hinoki" and also *Quercus acutissima*, a latifoliate tree which showed as vast distribution as "Hinoki" was collected at five locations in the mineralized district. Soil samples were also taken at those locations and analyzed. The results are shown in Table VIII. Acid-soluble and easily reducible manganese contents in the soils at the locations No. 4 and 5 which stand on or near the deposits are considerably high and the foliar analyses also seem to indicate the deposits. Both *Quercus* and "Hinoki" are applicable to the prospecting but the latter is better, because its leaves are available all the year round.

Summary

The possibility of practical application of foliar analyses for geochemical prospecting for manganese were examined. A positive correlation of manganese level between soil and plants was revealed by the experiments using water and soil culture.

For the practical application, a tree, *Chamaecyparis obtusa* was chosen to avoid small local variations, and the sampling variations due to age and position were determined.

The Mn/Fe ratio was found to be a more sensitive indicator than the manganese content itself and its average value in the mineralized district was 18.4; on the other hand, it was only 3.5 in non-mineralized district.

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*A New Analytical Method of Electroconductivity Data of Surfactant Solutions. II**

By Kenjiro MEGURO, Tamotsu KONDO and Noriaki OHBA

(Received December 15, 1956)

In the previous paper[1], a new analytical method for the electroconductivity data of aqueous solution of surfactant was reported. In this paper we apply this method to arrange and analyse the effect of temperature and of the addition of organic solvents and inorganic salts on the properties of surfactant solution.

The Effect of Temperature

It has been reported that the dependence of critical micelle concentration (C. M. C.) of surfactant solution on temperature is not evident; but it will be expected that some parameter which is related to the critical phenomena of surfactant solution will change with temperature.

According to our analysis of the electroconductivity data for dodecyl ammonium chloride at various temperatures, taken from Ralston[2], it is pointed out that the concentration of the minimum and the maximum in $(\Lambda_0-\Lambda)/\sqrt{c}$ vs. $\sqrt{c}$ curve and the slope between them do not vary considerably, but H_{min} and H_{max},—the magnitude of $\Lambda_0-\Lambda/\sqrt{c}$ at the minimum and the maximum—, vary conspicuously with temperature. The results are shown in Fig. 1.

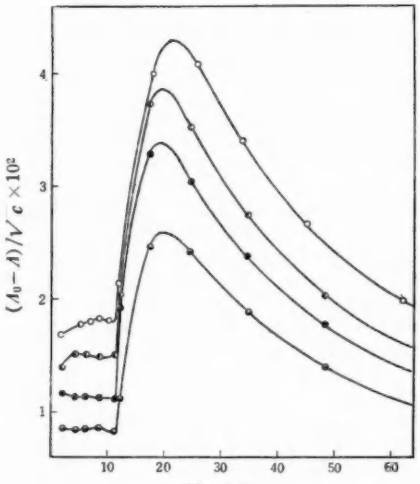

Fig. 1. $(\Lambda_0-\Lambda)/\sqrt{c}$ vs. $\sqrt{c}$ curves for the aqueous solution of dodecyl ammonium chloride at 20° ◒, 30° ●, 40° ◐ and 60°C ○.

The usual C. M. C. being remarkably invariant with temperature, H values might be of greater importance for the analysis of the effect of temperature on the critical phenomena of surfactant solution.

The Effect of Organic Solvent

From earlier data, it is noticed that the effects of addition of organic solvent upon the C. M. C. can be classified into those of lower fatty alcohols and of higher alcohols or hydrocarbons.

On the addition of a lower fatty alcohol, such as methyl or ethyl, the C. M. C. of surfactant shifts toward higher concentration with increasing alcohol content[3-5]. On the other hand, by the addition of a higher alcohol or a hydrocarbon, the C. M. C. is lowered[6-8], probably owing to solubilization. Such being the case, it is convenient to apply our analysis to each of two effects.

a) The Effect of Lower Fatty Alcohol: —Ward[3,4] and Kraus and his collaborators[5] measured the electroconductivity of sodium dodecyl sulfate and hexadecyl

* Presented at the 9th Annual Meeting of the Chemical Society of Japan held in Kyoto, April, 1956.

1) K. Meguro, T. Kondo, N. Ohba, et al., This Bulletin, **30**, 760 (1957).

2) A. W. Ralston, C. W. Hoerr and E. J. Hoffman, *J. Am. Chem. Soc.*, **64**, 97 (1942).

3) A. F. Ward, *Proc. Roy. Soc.*, **A176**, 412 (1940).

4) A. F. Ward, *J. Chem. Soc.*, **1939**, 552.

5) E. C. Evers and C. A. Kraus, *J. Am. Chem. Soc.*, **70**, 3049 (1948).

6) G. L. Brown, R. F. Grieger and C. A. Kraus, ibid., **71**, 95 (1949).

7) S. H. Herzfeld, M. L. Corrin and W. D. Harkins, *J. Phys. Coll. Chem.*, **54**, 971 (1950).

8) A. W. Ralston and D. N. Eggenberger, *J. Am. Chem. Soc.*, **70**, 983 (1948).

pyridonium bromide in water alcohol mixtures.

Our results by a new method are shown in Figs. 2 and 3. With increasing alcohol content of the solvent, the minimum and

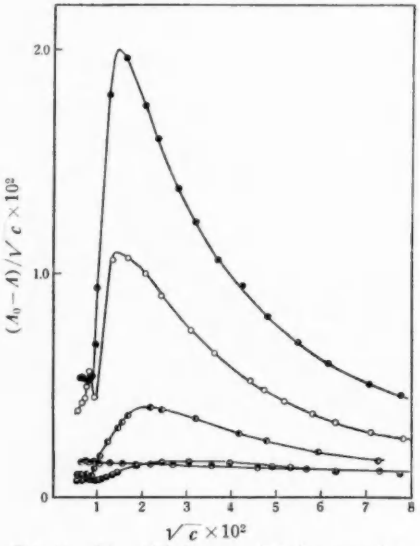

Fig. 2. $(\Lambda_0-\Lambda)/\sqrt{c}$ vs. $\sqrt{c}$ diagrams for sodium dodecyl sulfate in water-ethanol mixture: ● 0.0%, ○ 9.91%, ◐ 20.02%, ◒ 29.78%, and ◓ 39.60% of ethanol.

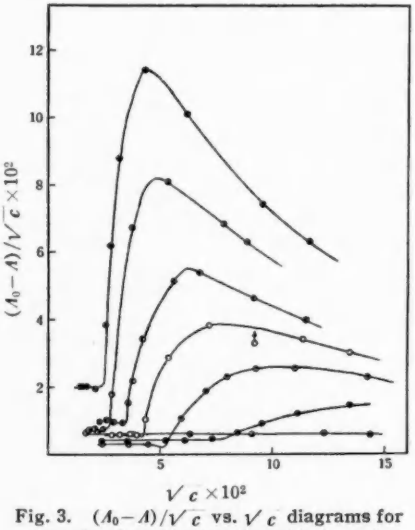

Fig. 3. $(\Lambda_0-\Lambda)/\sqrt{c}$ vs. $\sqrt{c}$ diagrams for hexadecyl pyridonium bromide in water-methanol mixture: ● 0.0%, ◑ 6.36%, ◐ 14.70%, ○ 19.91%, ◓ 26.02%, ● 35.20% and ◒ 54.14% of methanol.

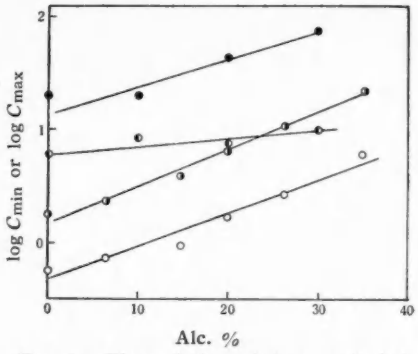

Fig. 4. The relations between alcohol content and C_{min} or C_{max} : for sodium dodecyl sulfate, ◐ C_{min} and ● C_{max} : for hexadecyl pyridonium bromide, ○ C_{min} and ◑ C_{max}.

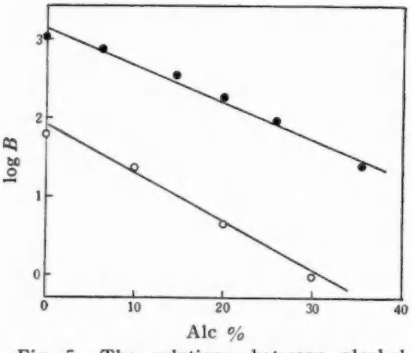

Fig. 5. The relations between alcohol content and log B; ○ sodium dodecyl sulfate and ● hexadecyl pyridonium bromide.

the maximum point shift toward higher concentrations, and the value of H_{max} decreases and at last the maximum disappears, while the slope between the minimum and the maximum becomes gradually gentle. From these data, we can find linear relations between log C_{min} or log C_{max} and alcohol content, between log H_{max} and alcohol content, and between log B and alcohol content as shown in Figs. 4 and 5 and Table I. (The meaning of the notations, C_{min}, C_{max} and B is the same as in the previous paper.)

b) The Effect of Solubilization :— Previously it was noted that the effect of the addition of higher alcohols or hydrocarbons on the C. M. C. was due to the solubilization of these substances in the micelle of surfactant. It is well known that there are two types in solubilization phenomena. One is of "palisade type",

TABLE I

THE CHANGE OF C_{min}, C_{max}, H_{min}, H_{max} AND B VALUES WITH ALCOHOL CONTENT

Substance	Alc. content %	C_{min}	C_{max}	H_{min}	H_{max}	B
$C_{12}H_{25}OSO_3Na$—EtOH	0.00	6.08	20.2	0.50	2.34	58.8
	9.91	8.50	20.2	0.45	1.10	23.6
	20.02	7.35	44.1	0.10	0.40	4.63
	29.78	9.90	75.8	0.08	0.16	0.98
$C_{16}H_{33}N^+$⟨⟩Br^-—MeOH	0.00	0.45	1.76	1.93	11.4	1080
	6.36	0.50	2.30	0.66	8.2	650
	14.70	1.16	3.85	0.95	5.5	350
$C_{18}H_{37}N^+$⟨⟩Cl^-—MeOH	0.00	0.24	1.06	0.30	11.0	1530
	9.90	0.56	2.03	0.83	6.80	700
	14.86	0.39	3.70	−0.73	4.52	333

TABLE II

Type of Solubilization	Effect	H_{min}	H_{max}	C_{min}	C_{max}
Palisade type	Increase in the amount of solubilizate	0	+	−	−
	Increase in the alkyl chain length of solubilizate	0	+	−	−
Sandwich type	Increase in the alkyl chain length of solubilizate	?	−	0	0

0: no remarkable change; +, increase; −, decrease

and the other of "sandwich type". The solubilization of higher alcohols belongs to the former type, and the solubilizates are situated in the micelle like palisades. On the other hand, the solubilization of nonpolar hydrocarbons, such as alkane and benzene, belongs to the latter type, and the solubilizates are situated, like contents of a sandwich, between surfactant molecules which construct a micelle.

If the difference between these types of solubilization could be observed by a suitable plotting of electroconductivity data, it would be very interesting, but it is impossible to do so by the usual plotting such as Λ vs. $\sqrt{c}$. According to our method, however, we can clearly distinguish them. In Fig. 6 and 7 is shown our analysis of palisade type solubilization, and in Fig. 8 that of sandwich type.

Comparing the effect of increase in the amount and in the alkyl chain length in the palisade layer on H_{min}, H_{max}, C_{min} and C_{max}, with the effect of increase in the alkyl chain length of solubilizates in the sandwich layer on the same values, we can obtain the results shown in Table II. The effects on these values, especially on H_{max} and C_{max}, form a good contrast between the palisade and the sandwich type. The fact that the effects of increase in the amount and in the alkyl chain length of solubilizates in the palisade layer correspond to those of increase in alkyl chain length of a surfactant molecule is suggestive of the existence of an intimate relation between them.

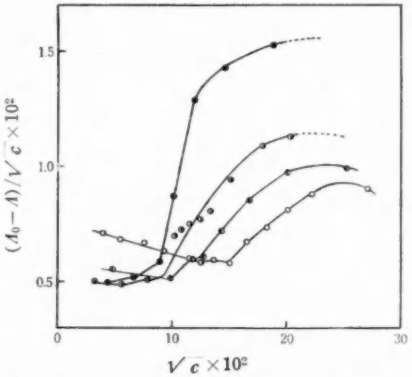

Fig. 6. $(\Lambda_0-\Lambda)/\sqrt{c}$ vs. $\sqrt{c}$ curves for dodecyl ammonium chloride in 24.65% methanol on addition of moles dodecanol per mole salt: ○ 0.0%, ◐ 0.110%, ◑ 0.261% and ● 0.651%.

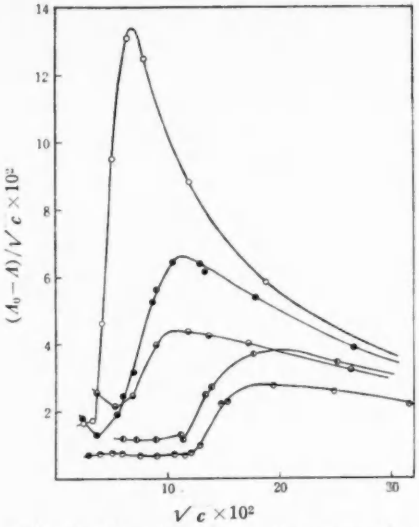

Fig. 7. $(\Lambda_0-\Lambda)/\sqrt{c}$ vs. $\sqrt{c}$ curves for dodecyl ammonium chloride solutions at 30°C in the presence of following substances; ◓ in water, ◒ hexanol, ● octanol, ○ dodecanol and ◐ octadecanol. (Adopted from Ref. 8)

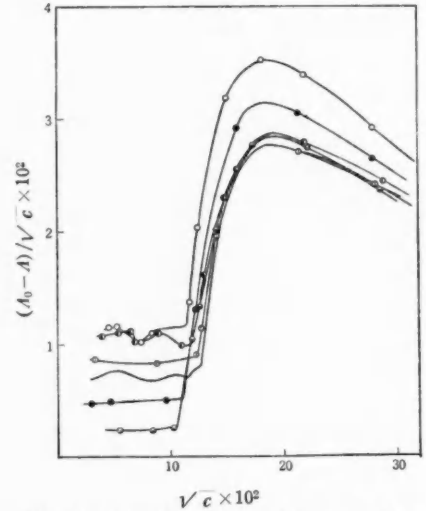

Fig. 8. $(\Lambda_0-\Lambda)/\sqrt{c}$ vs. $\sqrt{c}$ curves for dodecyl ammonium chloride solutions at 30°C in the presence of the following substances: (—) in water, ○ hexane, ● octane, ◒ dodecane, ◍ heptadecane and ◑ octadecane. (Adopted from Ref. 8)

The Effect of Added Salt

It is well known that the presence of an electrolyte affects considerably the behavior of aqueous solution of surfactants[9,10]. In general, the added salt promotes the micelle formation of surfactants. In order to apply our method to this case, Ralston's data[9] on the electroconductivity of aqueous solution of dodecyl ammonium chloride in 0.005 N and 0.015 N sodium chloride were used. The results obtained are shown in Fig. 9.

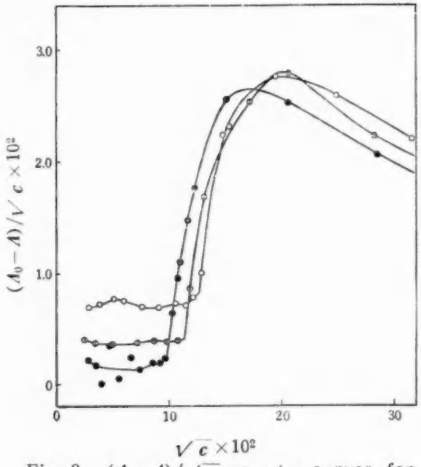

Fig. 9. $(\Lambda_0-\Lambda)/\sqrt{c}$ vs. $\sqrt{c}$ curves for dodecyl ammonium chloride in sodium chloride solutions: ○ 0.0 N, ◍ 0.005 N and ● 0.015 N.

The most remarkable thing is the occurring of influence upon H_{min} and no remarkable effect on H_{max} by the addition of salt, while C_{min} and C_{max} shift toward lower concentration with increasing concentration of the salt.

The authors wish to thank Professor B. Tamamushi, Tokyo University for his encouragement and advice in this work.

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9) A. W. Ralston and D. N. Eggenberger, ibid., **70**, 980 (1948).
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Polarographic Studies of Metallic Complexes. I. Oxalato-ammine, Glycinato-ammine and Ethylenediamine-ammine Series of Cobalt(III) Complexes[1]

By Nobufumi MAKI, Yoichi SHIMURA and Ryutaro TSUCHIDA

(Received May 23, 1957)

There have been reported some polarographic researches concerning the relation between the structures of cobalt(III) complexes and the half-wave potentials of their $Co^{III} \rightarrow Co^{II}$ reduction. For instance, Willis, Friend and Mellor[2] studied nitro-ammine series of cobalt(III) complexes and found the following order of the half-wave potentials:

$[Co(NO_2)_6]^{3-}$, $[Co(NH_3)_3(NO_2)_3]$, *cis*-$[Co(NH_3)_4(NO_2)_2]^+$, $[Co(NH_3)_2(NO_2)_4]^-$, *trans*-$[Co(NH_3)_4(NO_2)_2]^+$, $[Co(NH_3)_5NO_2]^{2+}$, $[Co(NH_3)_6]^{3+}$ (from positive to negative).

It was also reported by Brigando[3] that the polarographic stability of luteo-type cobalt(III) complexes increased as unidentate ligands were replaced by polydentate ligands. Thus the half-wave potentials of the luteo-type cobalt(III) complexes were in the order of

$[Co(NH_3)_4(NH_2CH_3)_2]^{3+}$, $[Co(NH_3)_6]^{3+}$, $[Co\ en_3]^{3+}$, $[Co\ dien_2{*}]^{3+}$.

Recently, Holtzclaw et al.[4-6] reported that the *cis* isomer exhibited a less negative half-wave potential than the corresponding *trans* isomer for the cobalt(III) complexes containing negative ligands on two of the coordination positions and neutral ligands on the other four. The complexes which contain only one or no negative ligands scarcely ever show such a trend.

The present paper deals with the polarography of oxalato-ammine, glycinato-ammine and ethylenediamine-ammine series of cobalt(III) complexes. The main purpose of this study is to examine the relation between the polarographic stabilities of these complexes and their structures.

Experimental

Apparatus.—A Heyrovsky-Shikata type polarograph was employed with the circuit for extendable and adjustable e.m.f. An H-cell with a saturated calomel electrode was used. The capillary used had an m-value of 5.828 mg./sec. and a drop time of 4.00 sec./drop in distilled water at the mercury height of 96 cm. with an open circuit. All measurements were carried out in a thermostat of 18.0 ± 0.1°C.

The half-wave potentials were determined from the enlarged polarograms by "plotting method". The potential values were calibrated for iR drop, using the minimum value of the cell resistance.

Materials.—The complex compounds studied were prepared by the method described in the literature[7-17] (the references are given in Tables I-III). The concentration of the solution was 0.001 gram complex ion per liter.

The supporting electrolytes used were potassium chloride, potassium sulfate or ammonium oxalate. The concentration of each supporting electrolyte was 0.1 F.

Oxygen was removed from the solutions for electrolysis by bubbling nitrogen gas through them for about half an hour, preventing the evaporation. Polarographic measurements were made as soon as possible after the removal of oxygen.

No maximum suppressor was used because it was feared that the presence of it might cause

1) Presented at the Tenth Annual Meeting of the Chemical Society of Japan, Tokyo, April 6, 1957.
2) J. B. Willis, J. A. Friend and D. P. Mellor, *J. Am. Chem. Soc.*, **67**, 1680 (1945).
3) J. Brigando, *Compt. rend.*, **236**, 708 (1953).
* dien=$NH_2CH_2CH_2NHCH_2CH_2NH_2$
4) H. F. Holtzclaw, Jr., *J. Am. Chem. Soc.*, **73**, 1821 (1951).
5) H. F. Holtzclaw, Jr., and D. P. Sheetz, ibid. **75**, 3053 (1953).
6) H. F. Holtzclaw, Jr., *J. Phy. Chem.*, **59**, 300 (1955).
7) "*Inorganic Syntheses*", **1**, 37 (1939).
8) E. H. Riesenfeld and R. Klement, *Z. anorg. Chem.*, **124**, 11 (1922).
* Polyoxyethylene sorbitan monooleate.
9) W. Schramm, ibid., **180**, 167 (1929).
10) T. Klobb, *Bull. soc. chim. France*, (3), **25**, 1025 (1901).
11) A. Werner and A. Vilmos, *Z. anorg. Chem.*, **21**, 145 (1899).
12) H. Ley and H. Winkler, *Ber.*, **42**, 3894 (1909).
13) Y. Shimura, to be reported later.
14) M. Linhard, *Z. Elektrochem.*, **50**, 224 (1944).
15) J. Meisenheimer, L. Angerman and H. Holsten, *Ann.*, **438**, 261 (1924).
16) Y. Shimura and R. Tsuchida, This Bulletin, **29**, 311 (1956).
17) A. Werner, *Ann.*, **386**, 1 (1912); A. Werner, F. Bräunlich, E. Rogowina and C. Kreutzer, ibid., **351**, 65 (1907).

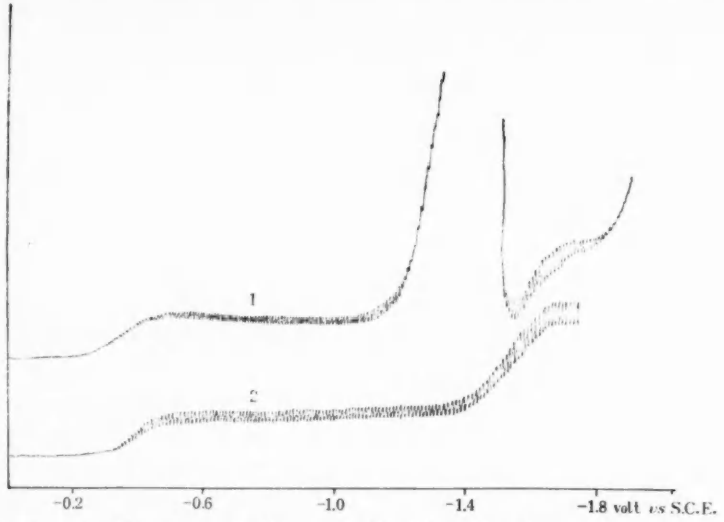

Fig. 1. (1) Polarogram of [Co en_2 gly]$Cl_2 \cdot H_2O$ in the absence of suppressor.
(2) Polarogram of [Co en_2 gly]$Cl_2 \cdot H_2O$ in the presence of 0.02% Tween-80.
Supporting electrolyte is 0.1 F KCl.

TABLE I

HALF-WAVE POTENTIALS OF OXALATO-AMMINE SERIES OF Co(III) COMPLEXES

(Supporting electrolyte: 0.1 F $(NH_4)_2C_2O_4$ or 0.1 F KCl).

Compound	First wave (volt *vs.* S. C. E.)		Second wave (volt *vs.* S. C. E.)	
	in $(NH_4)_2C_2O_4$	in KCl	in $(NH_4)_2C_2O_4$	in KCl
K_3[Co ox_3][7]	+		No reduction	
NH_4[Co$(NH_3)_2ox_2$]$\cdot H_2O$[8]	−0.03	−0.03	−1.58	−1.23
[Co$(NH_3)_4$ox]NO_3[9]	−0.21	−0.21	−1.46	−1.25
[Co$(NH_3)_6$]$SO_4 \cdot Cl \cdot 3H_2O$[10]	—	−0.24	—	−1.23
[Co en_2 ox]Cl[11]	−0.32	—	−1.35	—

TABLE II

HALF-WAVE POTENTIALS OF GLYCINATO-AMMINE SERIES OF Co(III) COMPLEXES

(Supporting electolyte: 0.1 F KCl or 0.1 F K_2SO_4)

Compound	First wave (volt *vs.* S. C. E.)		Second wave (volt *vs.* S. C. E.)	
	in KCl	in K_2SO_4	in KCl	in K_2SO_4
α-[Co gly_3][12]	−0.14	−0.07	*	*
[Co$(NH_3)_2$ gly_2]Cl[13]	−0.23$_0$	−0.28	−1.24	−1.31
[Co$(NH_3)_4$ gly]SO_4[13]	−0.23$_4$	−0.36	−1.26	*
[Co$(NH_3)_6$]$(ClO_4)_3$[14]	−0.24**	−0.43	−1.25**	−1.26
[Co en_2 gly]$Cl_2 \cdot H_2O$[15]	−0.36	−0.41	*	*

* Maximum wave

** The data of the chloride sulfate, [Co$(NH_3)_6$]$Cl \cdot SO_4 \cdot 3H_2O$.

the lowering of the limiting current and the shift of the half-wave potential to negative direction. For example, 0.02% Tween-80* shifted the half-wave potential of the first wave of glycinato-diethylenediamine cobalt(III) complex to the negative direction by −0.03 V (Fig. 1).

Results and Discussion

The half-wave potentials of the oxalato-ammine, glycinato-ammine and ethylenediamine-ammine series of cobalt(III)

complexes measured are given in Tables I-III.

TABLE III

HALF-WAVE POTENTIALS OF ETHYLENEDIAMINE-AMMINE SERIES OF Co(III) COMPLEXES

(Supporting electrolyte: 0.1 F KCl)

Compound	First wave (volt *vs.* S.C.E.)	Second wave (volt *vs.* S.C.E.)
$[Co\ en_3](ClO_4)_3$[14]	−0.43	*
cis-$[Co\ en_2(NH_3)_2](ClO_4)_3$[16]	−0.31	*
trans-$[Co\ en_2(NH_3)_2]Cl_3 \cdot H_2O$[17]	−0.31	*
$[Co\ en\ (NH_3)_4]_2(SO_4)_3 \cdot 4H_2O$[13]	−0.27	*
$[Co(NH_3)_6](ClO_4)_3$	−0.24	−1.23

* Maximum wave

All the compounds examined are reduced polarographically in two steps except in the case of the trioxalato complex, $[Co\ ox_3]^{3-}$, in the presence of an excess of $C_2O_4^{2-}$. On plotting $\log i/(i_d - i)$ against $E_{d.e.}$ for these three series of cobalt(III) complexes, straight lines were usually obtained. Only in a few cases, lines, having slight inflection were obtained. The reciprocal slopes of such lines for all the complexes showed that every reduction was irreversible.

The ratio of the height of first wave to that of the second is approximately 1 : 2. Consequently, it is concluded that the first wave, corresponding to a gain of one electron, apparently represents the reduction to cobalt(II) state, and the second, corresponding to two electrons, represents the reduction to the metal. The half-wave potential of the latter is always very near to that of hexaaquo cobalt(II) $[Co(OH_2)_6]^{2+}$ ion (−1.428 V *vs.* S.C.E. in 1 F K_2SO_4 solution[2]).

As regards the process of the reduction of hexammine cobalt(III) $[Co(NH_3)_6]^{3+}$, Laitinen et al.[18] offered the following mechanism:

$$[Co(NH_3)_6]^{3+} + e^- \longrightarrow [Co(NH_3)_6]^{2+}$$

$$[Co(NH_3)_6]^{2+} + 6H_2O \xrightarrow{\text{very rapid}} [Co(OH_2)_6]^{2+} + 6NH_3$$

$$[Co(OH_2)_6]^{2+} + 2e^- \longrightarrow Co + 6H_2O.$$

The similar mechanism of reduction is probably true for the oxalato-ammine and glycinato-ammine series of cobalt(III) complexes. In the case of the trisoxalato complex, $[Co\ ox_3]^{3-}$, no reduction wave is observed below the final current rise in solutions containing an excess of oxalate ion. This fact has already been reported by Watters and Kolthoff[19]. This is probably an indication of the presence of a stable cobalt(II) oxalato-complex in the solution such as dioxalatodiaquo cobaltate (II) $[Co\ ox_2(OH_2)_2]^{2-}$ or a similar one.

All the second waves of the ethylenediamine-ammine complexes have maxima, which can be easily suppressed by a capillary active substance. According to Kivalo[20], the product of the first reduction of trisethylenediamine cobalt(III) $[Co\ en_3]^{3+}$ is an equilibrium mixture of aquo-ethylenediamine cobalt(II) complexes. The second waves of the ethylenediamine-ammine cobalt(III) complexes are, therefore, due to the similar aquo-ethylenediamine cobalt(II) complexes.

As to the relation between the half-wave potential of the first wave of these complexes and their structure, the authors are led to the following conclusion. As the ammonia molecules in hexa-ammine cobalt(III) $[Co(NH_3)_6]^{3+}$ are replaced with ethylenediamine, the half-wave potential of the first wave shifts to the negative direction. If the ammonia molecules in $[Co(NH_3)_6]^{3+}$ are replaced by glycinate the first wave shifts to the positive direction. similarly, by successive replacements of the ammonia molecules in $[Co(NH_3)_6]^{3+}$ with oxalate, the first wave shifts to further more positive. Since the half-wave potential of the first wave of trisoxalato cobaltate(III) $[Co\ ox_3]^{3-}$, a chelate produced by exhaustive replacement with oxalate, is in the positive region of applied potential *vs.* S.C.E., it is hardly possible to measure its value at

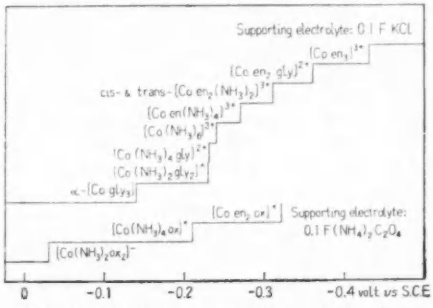

Fig. 2. Half-wave potentials of the first wave of oxalato-ammine, glycinato-ammine and ethylenediamine-ammine series of Co(III) complexes.

18) H. A. Laitinen, J. C. Bailar, Jr., H. F. Holtzclaw and J. V. Quagliano, *J. Am. Chem. Soc.*, **70**, 2999 (1948).

19) J. I. Watters and I. M. Kolthoff, *Anal. Chem.*, **21**, 1466 (1949).

20) P. Kivalo, *J. Am. Chem. Soc.*, **77**, 2678 (1955).

the dropping mercury electrode.

Eventually, the polarographic stability of cobalt(III) complexes increases in the following order of the ligands (see Fig. 2): ox^{2-}, gly^{-}, NH_3, en. The half-wave potential of $[Co\ en_2\ gly]^{2+}$ is more negative than that of $[Co\ en_2\ (NH_3)_2]^{3+}$. This is an exception of the order above mentioned. As is also seen from the work of Willis et al.[2] on the nitro-ammine cobalt(III) complexes, it is suggested that many factors affect the position of the first wave of cobalt(III) complexes.

In agreement with the observation of Holtzclaw et al.[5], *cis*- and *trans*-forms of $[Co\ en_2(NH_3)_2]^{3+}$ have identical values of the half-wave potential of the first wave.

Summary

The ions $[Co\ ox_n(NH_3)_{6-n}]^{(2n-3)-}$, $[Co\ gly_n(NH_3)_{6-n}]^{(3-n)+}$, $[Co\ en_n(NH_3)_{6-n}]^{3+}$, $[Co\ en_2\ gly]^{2+}$ and $[Co\ en_2\ ox]^{+}$ were all reduced irreversibly in two steps at the dropping mercury electrode.

The first diffusion current corresponds to the reduction, $Co^{III} \rightarrow Co^{II}$ and the second to the reduction, $Co^{II} \rightarrow Co^{0}$. The diffusion current and the concentration were linearly related.

The half wave potential of the first wave of the cobalt(III) complexes shifts to negative direction in the following order of the ligands: ox^{2-}, gly^{-}, NH_3, en.

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Further Studies on the Degradation of Yeast Ribonucleic Acid by Lanthanum Hydroxide

By Masazumi MIYAZAKI

(Received May 13, 1957)

By applying lanthanum hydroxide, which E. Bamann[1] introduced as a phosphatase model, to ribonucleic acid (RNA), F. Egami and M. Shimomura[2,3] found that RNA was almost completely hydrolysed to nucleosides and inorganic phosphate. This finding was confirmed and extended by E. Bamann[4,5], K. Dimroth[6], F. W. Allen[7] and their coworkers. The present paper deals with further studies on the degradation of yeast ribonucleic acid by lanthanum hydroxide gel with special reference to the nature of the products and the order of their formation.

Experimental

Materials and Solvent Systems.—*Yeast ribonucleic acid.*—A commercial RNA (Schwarz Laboratories) was used as such or after purification by the chloroform gel method[8]. The phosphorus content of the purified preparation was 8.5% and that of the original RNA about 8.0%.

LaRF.—M. Shimomura[9] found that a part of RNAwas resistant to lanthanum hydroxide. He named this fraction "lanthanum hydroxide resistant fraction (LaRF)". It was prepared according to Shimomura[9].

Solvent Systems for Paper Chromatography.—Solvent system 1[10], 70% (vol.) isopropanol in concentrated aqueous ammonia in the bottom of the chamber (0.35 ml for each liter of gas space); descending for 17 to 18 hr.; suitable for fractionation of various oligo- and mononucleotides.

1) E. Bamann, *Angew. Chem.*, **52**, 186 (1939).
2) F. Egami and M. Shimomura, *Science (Japan)*, **18**, 472 (1948).
3) M. Shimomura and F. Egami, This Bulletin, **26**, 263 (1953).
4) E. Bamann, F. Fischler and H. Trapmann, *Biochem. Z.*, **325**, 413 (1954).
5) E. Bamann, H. Trapmann and F. Fischler, *Biochem., Z.*, **326**, 89 (1954).
6) K. Dimroth, L. Jaenicke and D. Heinzel, *Ann. Chem.*, **566**, 206 (1950). K. Dimroth, L. Jaenicke and D. Heinzel, *Z. Physiol. Chem.*, **289**, 71 (1952).
7) J. E. Backer and F. W. Allen, *J. Biol. Chem.*, **188**, 59 (1951).
8) M. G. Sevag, D. B. Lackman and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).
9) M. Shimomura, This Bulletin, **27**, 29 (1954).
10) R. Markham and J. D. Smith, ibid., **52**, 552, 558, 565 (1952).

Solvent system 2, 5 ml. of concentrated aqueous ammonia added to 100 ml. of 86% (vol./vol.) aqueous *n*-butanol; descending for 17 to 18 hr.; useful for separation of the four nucleosides.

Solvent system 3[11], a mixture of saturated ammonium sulfate in water (79 ml.), water (19 ml.) and isopropanol (2 ml.); ascending for 7 hr.; used for separation of 2'- and 3'-purine nucleotides.

Solvent system 4[12], a mixture of isopropanol (170 ml.), concentrated hydrochloric acid (41 ml.) and water (total 250 ml.); ascending for 36 hr.; used for separation of adenine, guanine, cytidylic and uridylic acid.

Process of Degradation of RNA by Lanthanum Hydroxide.—The lanthanum hydroxide suspension was made by dissolving lanthanum carbonate with hydrochloric acid and then by bringing the pH of the solution to the alkaline side. When the RNA solution was mixed with the lanthanum hydroxide suspension, RNA was bound completely to lanthanum hydroxide in the presence of an excess of the latter as shown in Figs. 1, 2, 3, and in Tables II and III, where 2 g. of lanthanum carbonate was used per gram of RNA. When 0.35 g. of the carbonate was used per gram of RNA, this being the condition in experiments for Fig. 4 and Table I, some amount of RNA was left unbound. Nucleosides, which were liberated during the reaction, were not adsorbed on lanthanum hydroxide, whereas liberated inorganic phosphate was bound to lanthanum hydroxide.

Fractionation of Oligo- and Mononucleotides formed from RNA by Lanthanum Hydroxide.—At intervals, the precipitates containing nucleotides and phosphate were separated from the reaction mixture by centrifuge and washed with hot water. They were extracted with 5% sodium bicarbonate solution and fractionated by paper chromatogram using solvent system 1. Thus a chromatogram shown in Fig. 1. was obtained. The reaction was carried out under the following conditions: pH 8.5 and 60°C, 500 mg. RNA and 1g. lanthanum carbonate.

By analysing the base constituents and comparing the spots with those of mononucleotides, it was suggested that fraction A contained pyrimidine nucleotides and adenylic acid, fraction B guanylic acid and oligonucleotides and fractions C, D and E polynucleotides. Under more drastic conditions, at 80°, a few hours were sufficient to degrade RNA to fractions A and B. When 10—20% of the original RNA was dephosphorylated, most of products were composed of fractions A and B.

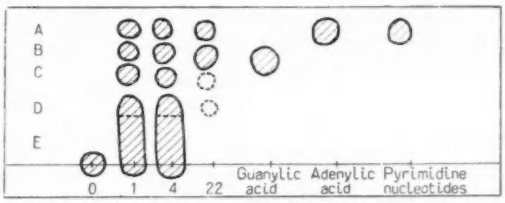


Fig. 1. Fractionation of lanthanum hydroxide hydrolysates by paper chromatography.

On the other hand, degradation of RNA by a less amount of lanthanum hydroxide (lanthanum carbonate 70 mg. to RNA 200 mg., that is, the atomic ratio of lanthanum to RNA phosphorus was 1/4), proceeded as shown in Table I.

Although the supernatant contained RNA, dephosphorylation did not occur in this fraction, but only in the precipitate fraction. On the other hand, paper chromatography with solvent system 1 showed that RNA in both the supernatant and the precipitate was similarly degraded to fractions A and B within 12 hr.

TABLE I

DEPHOSPHORYLATION OF RNA BY A LESS AMOUNT OF LANTHANUM HYDROXIDE. THE REACTION WAS CARRIED OUT AT 60° FOR 9 HR. AND CONTINUED AT 80°. THE WHOLE REPRESENTS THE ENTIRE REACTION MIXTURE AND, AT INTERVALS, WAS DIVIDED INTO THE SUPERNATANT CONTAINING UNBOUND RNA AND THE PRECIPITATE

Fraction	Time (hr.)			
	5	12	27	51
Supernatant	0%	0%	0%	0%
Whole	ca. 0	3.3	18	31
Precipitate	ca. 0	12	49	74

Production of 2'- and 3'-Purine Nucleotides.—The precipitates, which were separated from the reaction mixture by centrifuge and washing, were extracted with 5% sodium carbonate solution. By two dimensional paper chromatography with solvent system 1 and 3, 2'- and 3'-adenylic, and 2'- and 3'-guanylic acid were detected in the extracts.

According to this chromatogram, 2'-purine nucleotides were produced less than the corresponding 3'-nucleotides, and

11) R. Markahm and J. D. Smith, ibid., **49**, 401 (1951).
12) G. R. Wyatt, ibid., **48**, 584 (1951).

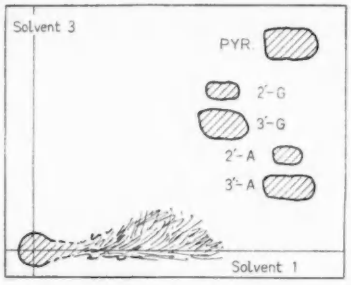


Fig. 2. Two dimensional paper chromatogram

A proviso: RNA 500 mg. lanthanum carbonate 1 g. at pH 8.5 and 80, after 1 hr.

Spots: PYR., 2'-G, 3'-G, 2'-A, 3'-A, are abbreviations for pyrimidine nucleotides, 2'- and 3'-guanylic, 2'- and 3'-adenylic acid, respectively.

this tendency was still more markedly observed in the precipitate fraction under the same conditions as in Table I. Besides we were not successful in detecting 5'-mononucleotides by the Boeseken reaction and the periodate oxidation method[13].

Liberation of Nucleosides.—Purine-bound ribose was measured by the orcinol reaction on the assumption that the color intensity was given quantitatively and only by purine-bound ribose. The molar ratio of purine-bound ribose/RNA-phosphorus of the original RNA preparation was taken as 0.5, assuming that equimolar proportions of purine and pyrimidine nucleotides were present.

As shown in Fig. 3, the ratio increased with reaction time. It seems to indicate that there is an increase in the amount of purine-bound phosphorus in the undephosphorylated RNA; it is evident that pyrimidine nucleosides were liberated faster than purine nucleosides on the whole (Fig. 3).

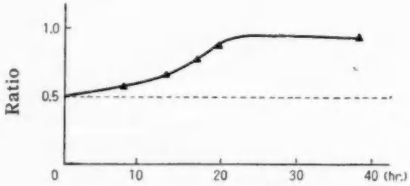


Fig. 3. Change in the purine-bound ribose/RNA-phosphorus ratio of the undephosphorylated RNA with time under the same conditions as used in Fig. 2.

Interrelation between purine nucleosides and also between pyrimidine nucleosides was observed as in the following Tables IIa and IIb. Adenosine was liberated faster than guanosine, and cytidine faster than uridine as expected from the dephosphorylation of mononucleotides[5] (Table III). However unexpected liberation of nucleosides was observed at the initial stage of reaction. Namely, at the initial stage liberation of adenosine was extraordinarily more than that of guanosine, and that of uridine was faster than that of cytidine, and increased later again during the reaction.

TABLE IIa

LIBERATION OF PURINE NUCLEOSIDES AT VARIOUS INTERVALS, THE MOLAR RATIO TAKING GUANOSINE AS UNITY

Nucleosides	Time (hr.)			
	0 to 0.5	0.5 to 6	6 to 20	20 to 40
Guanosine	1	1	1	1
Adenosine	2.5	1.7	0.5	0.3

TABLE IIb

LIBERATION OF PYRIMIDINE NUCLEOSIDES AT VARIOUS INTERVALS, THE MOLAR RATIO TAKING URIDINE AS UNITY

Nucleosides	Time (hr.)			
	0 to 0.5	0.5 to 6	6 to 20	20 to 40
Uridine	1	1	1	1
Cytidine	0.6	1.3	0.9	0.6

TABLE III

DEPHOSPHORYLATION OF MONONUCLEOTIDES UNDER THE FOLLOWING CONDITIONS: pH 8.5 AND 80°, EMPLOYING MONONUCLEOTIDES 30 mg. EACH AND LANTHANUM CARBONATE 200 mg.

Nucleotide	Time (hr.)		
	5	20	40
Guanylic acid	39%	90%	100%
Adenylic acid	55	94	100
Uridylic acid	40	93	100
Cytidilic acid	70	97	100
5'-Adenylic acid	12	54	100

The preferential liberation of uridine in the beginning was clearly observed on the chromatogram of the nucleosides in the supernatant of the reaction mixture under the same conditions as in Table I. In this experiment, the atomic ratio of lanthanum to RNA phosphorus was about 1/4 (Fig. 4). For the first 5 hr., uridine was detected as the only nucleoside on

13) K. Makino, *J. Japan. Biochem. Soc.*, **27**, 489 (1955).

the chromatogram; it was observed that, at this time, little of RNA was dephosphorylated and only a small amount of pyrimidine nucleotides was detected on the chromatogram with solvent system 3.

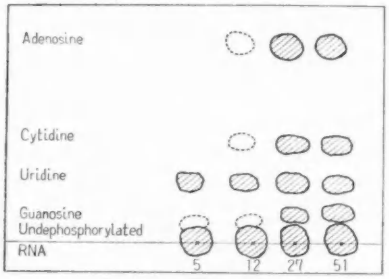


Fig. 4. Nucleosides chromatogram with solvent system 2

On the Nature of LaRF.—After dialysis of LaRF against distilled water through a cellophane bag for 2 days, the dialysed fraction showed the following broad absorption curve with maximum about 260 mμ; however the nondialysable fraction had no absorption maximum at 260 mμ (Fig. 5). The latter fraction did not give the orcinol reaction either.

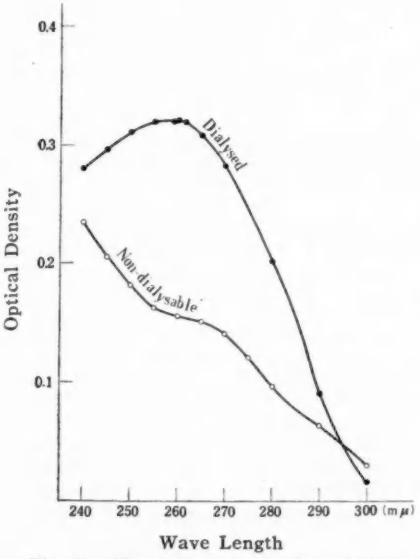


Fig. 5. Absorption curve after dialysis

LaRF at the concentration of 4 to 5 mg./cc. was not precipitated with uranium reagent (0.25% uranyl acetate in 2.5% aqueous trichloroacetic acid solution; this is a powerful precipitant of oligo- and polynucleotides) and by lowering pH to 1 by hydrochloric acid, either.

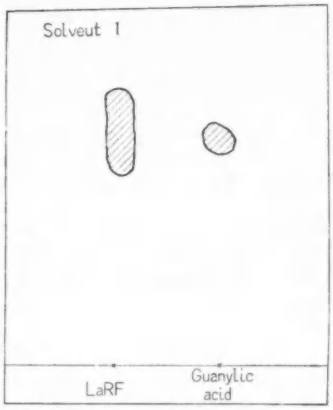


Fig. 6. Paper chromatogram of LaRF

Paper Chromatography of LaRF.—Fig. 6 shows the paper chromatogram of LaRF with solvent system 1. The R_f value of LaRF was close to that of guanylic acid and no spots were detected near the origin. So LaRF seems to contain no large polynucleotides. The spot of LaRF on this chromatogram showed fluorescence by hydrogen chloride fume. Molar ratios of bases in LaRF:

TABLE IV
MOLAR RATIOS OF BASES IN LaRF WHERE GUANINE IS UNITY

Guanine	Adenine	Uracile	Cytosine
1	0.8	ca. 0.2	ca. 0.3

According to this analysis of the base constituents, LaRF contained predominantly guanine, and adenine and a much smaller amount of uracil and cytosine as already suggested by Shimomura[9].

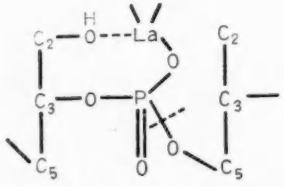
Discussion

The formation of four mononucleotides at the early stage of reaction (10~20% dephosphorylation) and the parallelism between the liberation of nucleosides and the dephosphorylation velocity of mononucleotides seem to indicate that most of the RNA was degraded to mononucleotides, and the latter were in turn dephosphorylated.

However it should be noted that the liberation of uridine and adenosine was unexpectedly fast at the beginning and

that the liberation of uridine increased later again. These findings seem to suggest that lanthanum hydroxide binds more easily with certain specific positions of RNA, and uridine exists in two different positions.

As intermediate reaction products, 2'- and 3'-purine nucleotides were detected. 2'-Nucleotides were less in amount than corresponding 3'-nucleotides, especially in the experiments with a small quantity of lanthanum hydroxide. These facts may be explained by Bamanns inner complex hypothesis[5].



Concerning LaRF, its chemical nature could not be finally elucidated. However it was concluded that it is a low molecular weight compound with high guanylic and adenylic acid content. It is not yet resolved, on what its resistance to lanthanum hydroxide depends.

Summary

The degradation of yeast ribonucleic acid by lanthanum hydroxide was reinvestigated. The results may be summarized as follows:

1) Most of the RNA seems to be degraded through mononucleotides to nucleosides and inorganic phosphate.

2) 2'- and 3'-purine nucleotides were detected as intermediate products. 2'-Nucleotides were produced in less amounts than the corresponding 3'-nucleotides.

3) On the whole, pyrimidine nucleosides were liberated faster than purine nucleosides.

4) Liberation of uridine was characteristic. It was preferencially liberated at the beginning and its liberation predominated again at the last stage of reaction.

5) "Lanthanum hydroxide resistant fraction (LaRF)" was dialysable, not precipitated with uranium reagent and had R_f value close to that of guanylic acid.

The author wishes to thank Professor Fujio Egami and Dr. Michio Shimomura for their useful discussion as well as their constant encouragement in the course of the work. The expense of this study was defrayed in part by a grant from the Ministry of Education.

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Molecular Rotations of Glucides in Relation to their Structures. III. Optical Superposition in Pentoses

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(Received May 14, 1957)

In 1874, vant't Hoff suggested that the partial rotation contributed by a given asymmetric carbon atom to the total rotation of a molecule containing several such atoms, is independent of the configuration of these other atoms*1. He predicted also that the rotation of arabinose should be equal to the sum of those of xylose, ribose and the expected fourth type of pentose at that time (lyxose), from the standpoint of the orientations of OH groups in their open chain structure[1]. His prediction, however, was proved to be invalid by many experiments. Hudson found some interesting facts, suitable for proving van't Hoff's principle of optical superposition*1 in glucides, and he put forward two

*1 Guye and Gautier (Ph. A. Guye and M. Gautier, *Compt. rend.*, **119**, 740 (1894)) named this idea "principe de la superposition algébrique des effets optiques des carbones asymétriques". This is called generally "vant't Hoff's principle of optical superposition".

1) Van't Hoff, *Bull. Soc. Chim.*, **23**, 298 (1875).
2) C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

TABLE I

Name	Unit Groups	$[M]_D^{20}(W)$	Lit.
β-L-arabinose	[$(OH)^{1\alpha}$, $(OH)^{2\alpha}$, $(OH)^{3\beta}$, $(OH)^{4\beta}$, Ring]	303.3°	(a)
α-L-arabinose	[$(OH)^{1\beta}$, $(OH)^{2\alpha}$, $(OH)^{3\beta}$, $(OH)^{4\beta}$, Ring]	133.6°	(a)
α-D-xylose	[$(OH)^{1\alpha}$, $(OH)^{2\alpha}$, $(OH)^{3\beta}$, $(OH)^{4\alpha}$, Ring]	138.1°	(b)
β-D-xylose	[$(OH)^{1\beta}$, $(OH)^{2\alpha}$, $(OH)^{3\beta}$, $(OH)^{4\alpha}$, Ring]		
α-D-ribose	[$(OH)^{1\alpha}$, $(OH)^{2\alpha}$, $(OH)^{3\alpha}$, $(OH)^{4\alpha}$, Ring]		
β-D-ribose	[$(OH)^{1\beta}$, $(OH)^{2\alpha}$, $(OH)^{3\alpha}$, $(OH)^{4\alpha}$, Ring]		

isorotation rules as set out below[2]:

Rule 1—"The rotation of C^1- atom in the case of many substances of the sugar group is affected in only a minor degree by changes in the structure of the remainder of the molecule".

Rule 2—"Changes in the structure of C^1- atom in the case of many substances of sugar group affect in only a minor degree the rotation of the remainder of the molecule".

But mannose deviates from the first empirical rule. Then, in order to explain this anormality, Hudson assigned to α-D-mannose a five-membered (furanose) ring, whilst admitting the presence of the six-membered (pyranose) ring in glucose and galactose, as well as in β-D-mannose[3]. Nevertheless, his idea was not supported by any independent chemical evidence. In 1929, under the hypothesis that the van't Hoff's principle exists in glucide, that is to say, every one of the asymmetric carbon atoms in the aldopyranose-ring possesses its own definite partial molecular rotation, Isbell calculated the values of these partial molecular rotations by simple algebraic summation and subtraction of $[M]_D^{20}(W)$*2 of several glucides. By using these calculated values, he presumed $[M]_D^{20}(W)$ of sixteen glucides, then unknown[4]. However, many independent experiments have proved most of them to be inaccurate. Haworth and Hirst attributed these irregularities of rotatory power to the influence of dissimilarities of configuration[5], in accordance with the view of Rosanoff[6] who held that the optical rotatory power of an asymmetric carbon atom depends not only upon the composition and constitution, but also upon the configuration of the glucides. Rosanoff's view was very splendid. But it had no ground of physical theory and was not quantitative. In 1951, the author proposed the application of the calculation-method based on quantum mechanics, to illustrate $[M]_D^{20}(W)$ of glucides[7]. Lately, the author succeeded in explaining Hudson's first isorotation rule and the deviation of mannose from this[8], on the basis of Kirkwood's polarizability theory of optical rotatory power[9], which adopted the idea that the partial rotation is caused by the dynamical coupling effect between any two unit groups in a molecule.

By using the method which is similar to that used in the previous paper[8], the author tried here to elucidate the relation between the structure of pentoses*3 and the value of their molecular rotations; this attempt had been continued since the time of van't Hoff without success, and it was found that there exists the principle of optical superposition of Kirkwood's idea in the pentose group.

Molecular Model and Data Used*4

The names of pentoses, to which the calculations were applied, unit groups in their molecules, and their corresponding molecular rotations are tabulated in Table I*5.

In order to simplify the calculations, the molecular model of an aldopyranose-ring of Reeves' C 1 conformation (the bond length of C–O is 1.42 Å) which was used in the previous paper[8], was also adopted here. (Fig. 1)

The optical data for OH group and H

3) C. S. Hudson, *J. Am. Chem. Soc.*, **52**, 1680 (1930).

*2 $[M]_D^{20}(W)$ is a notation which represents the optical molecular rotation of the plane polarized light of wave-length of D line at 20°C by an aqueous solution.

4) H. S. Isbell, *Bureau of Standards. J. Res.*, **3**, 1041 (1929).

5) W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, **1930**, 2615.

6) M. A. Rosanoff, *J. Am. Chem. Soc.*, **28**, 525 (1906); **29**, 536 (1907).

7) S. Yamana, Presentation and Discussion at the XIIth International Congress of Pure and Applied Chemistry. September (1951), New York.

8) S. Yamana, This Bulletin, **30**, 207 (1957).

9) J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).

*3 In the cases of hexose and methyl pentose, there are CH_2OH and CH_3 groups which combine with C^5-atom of the aldopyranose-ring, respectively. Then, the calculations become very complicated. In order to avoid this intricacy, only the pentose group is treated in this article. (Concerning the problem of hexose and methyl pentose, refer to Part IV of this study.

*4 Abbreviated symbols and notations, used in this paper, are the same as those in the previous paper (8).

*5 According to Reeves' continued investigations of cuprammonium-glycoside complexes (R. E. Reeves, *J. Am. Chem. Soc.*, **72**, 1499 (1950)), all of the following pentoses are of the C1 conformation of ring structure in their aqueous solutions, but the one remaining pentose (lyxose) is of both C1 and 1C conformations in its aqueous solution. Reeves, however, did not speak of the value of the quantitative ratio between these two types of conformation. Accordingly, it is impossible to try to make the calculation on lyxose in this paper.

a) E. Montogomery and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 2074 (1934).

b) J. K. Wolfe, R. M. Hann and C. S. Hudson, ibid., **64** 1493 (1942).

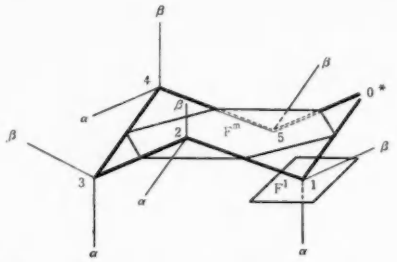

Fig. 1. Perspective drawing of the molecular model of an aldopyranose-ring of Reeves' C 1 conformation.
F^m.....(Flar)^m-plane
F^1.....(Flar)^1-plane

atom, used in the previous paper[10], were employed again without any correction. The refractive indices of the aqueous solutions of pentoses under question are presumed to be nearly equal (1.34)*6.

Method Proposed

The principle of the method of calculation is similar to that used in the previous paper[8]. The outline of this method is as follows;

(i)—The molecular model of pentose was divided into unit groups*7.

(ii)—$[\mu]^{20}_{D\text{calcd}}(3/n^2+2)$, caused by the dynamical coupling effect between any two members of these unit groups were calculated according to Kirkwood's polarizability theory*8.

(iii)—$[\mu]^{20}_{D\text{obs}}$ was calculated from $[\mu]^{20}_{D\text{calcd}}(3/n^2+2)$, above obtained*9.

(iv)—In order to avoid the difficulty, caused by the impossibility of calculation of the terms with which the aldopyranose-ring is concerned, quasi-empirical methods were devised by using the experimental data of $[M]^{20}_{D}(W)$ of β-L-arabinose, α-L-arabinose and α-D-xylose.

(v)—By algebraic summation of $[\mu]^{20}_{D\text{obs}}$s, above obtained, $[M]^{20}_{D}(W)$s of β-D-xylose, α-D-ribose and β-D-ribose were calculated.

(vi)—These calculated values were compared with the molecular rotations which have been observed under such conditions as close to $[M]^{20}_{D}(W)$ as possible.

TABLE II

$[\mu]^{20}_{D\text{obs}}$, CAUSED BY THE DYNAMICAL COUPLING EFFECT BETWEEN ANY TWO MEMBERS OF UNIT GROUPS (EXCEPT RING)

	4,β	4α	3β	3α	2,β	2α
1α	0	0	6.8	0	0	26.8
1β	0	0	0	− 6.8	26.8	−26.8
2α	6.8	0	26.8	−26.8		
2β	0	− 6.8	−26.8	0		
3α	0	26.8				
3β	26.8	−26.8				

This method of calculation is illustrated by an example. The $[\mu]^{20}_{D\text{obs}}$, caused by dynamical coupling effect between any two members of unit groups in α-D-xylose molecule, are*7 (1α)ƛ(2α)*10, (1α)ƛ(3β), (1α)ƛ(4α), (1α)ƛR*11, (2α)ƛ(3β), (2α)ƛ(4α), (2α)ƛR, (3β)ƛ(4α), (3β)ƛR and (4α)ƛR. Then, $[M]^{20}_{D}(W)$ of α-D-xylose, 138.1[b], should be equal to the sum of these $[\mu]^{20}_{D\text{obs}}$s*12. This relation is given by the following equation. (ref. Table II)

$$\begin{aligned}138.1 \equiv \sum[\mu]^{20}_{D\text{obs}} &= 26.8+6.8+0 \\ &+(1\alpha)\text{ƛR}+26.8+0+(2\alpha)\text{ƛR} \\ &-26.8+(3\beta)\text{ƛR}+(4\alpha)\text{ƛR} \\ &=33.6+(1\alpha)\text{ƛR}+(2\alpha)\text{ƛR} \\ &+(3\beta)\text{ƛR}+(4\alpha)\text{ƛR} \qquad (1)\end{aligned}$$

Owing to the symmetry of the aldopyranose-ring,

$$(3\beta)\text{ƛR} \equiv 0 \qquad (2)$$

10) S. Yamana, This Bulletin, **30**, 203 (1957).
*6 Ref. *8 in the previous paper (8).
*7 Ref. Table 1 in this article.
*8 Concerning the method of calculation, refer to the previous paper (10).
*9 According to the previous paper(8), $[\mu]^{20}_{D\text{calcd}}$, caused by the dynamical coupling effect between two OH groups in an aldopyranose molecule of C1 conformation in aqueous solution, becomes equal to $[\mu]^{20}_{D\text{obs}}$, when multiplied by $\kappa=12.37/n^2+2$. Then, in order to obtain $[\mu]^{20}_{D\text{obs}}$ from $[\mu]^{20}_{D\text{calcd}}(3/n^2+2)$, the latter must be multiplied by $(n^2+2/3)\ (12.37/n^2+2)=12.37/3=4.123$. Consequently, the table of $[\mu]^{20}_{D\text{obs}}$, caused by the dynamical coupling effect between any two OH groups in this case, can be made by multiplying the values of $[\mu]^{20}_{D\text{calcd}}(3/n^2+2)$ in Table I of the previous paper (8) with this coefficient, 4.123. But, in order to obtain the first significant decimal figures of $[\mu]^{20}_{D\text{obs}}$ more precisely, the absolute values, 1.66 and 6.50, should be used instead of 1.7 and 6.5 of the previous paper, respectively. It is important that the coefficient 4.123 was obtained by using the data of $[M]^{20}_{D}(W)$ of four hexoses(α-D-glucose, β-D-glucose, α-D-mannose and β-D-mannose), indifferent to those of pentoses.
*10 The notation X was used in the previous papers (10) (8) to represent $[\mu]^{20}_{D\text{calcd}}(3/n^2+2)$, caused by the dynamical coupling effect between two unit groups. But, in this paper, $[\mu]^{20}_{D\text{obs}}$ is used instead of $[\mu]^{20}_{D\text{calcd}}(3/n^2+2)$ for the algebraic summation course. Then, the other notation becomes necessary. The symbol, ƛ, was devised for this reason. This is inverted Y [wai] and is called "iaw [iɔ:]". $(1\alpha)\overset{20}{\underset{D}{\text{ƛ}}}(2\alpha)$ means $[\mu]^{20}_{D\text{obs}}$, caused by the dynamical coupling effect between $(OH)^{1\alpha}$ and $(OH)^{2\alpha}$. But 20 and D in $\overset{20}{\underset{D}{\text{ƛ}}}$ were omitted generally for convenience.
*11 R is an abbreviated symbol of Ring.
*12 Ref. Eq. (3) in the previous paper (10).

and $(2\alpha)\Delta R+(4\alpha)\Delta R\equiv 0$ (3)

Combining these two conditional equations with Eq. (1),

$$(1\alpha)\Delta R=104.5 \quad (4)$$

A similar treatment was applied also to the cases of β-L- and α-L-arabinose, and the following two equations were obtained. From β-L-arabinose,

$$(1\alpha)\Delta R+(2\alpha)\Delta R+(4\beta)\Delta R=209.3 \quad (5)$$

and from α-L-arabinose,

$$(1\beta)\Delta R+(2\alpha)\Delta R+(4\beta)\Delta R=100.0 \quad (6)$$

By subtracting Eq. (4) from (5),

$$(2\alpha)\Delta R+(4\beta)\Delta R=104.8 \quad (7)$$

and by subtacting Eq. (7) from (6),

$$(1\beta)\Delta R=-4.8 \quad (8)$$

Thus, the values of two $[\mu]^{20}_{D\,obs}$s, $\{(1\alpha)\Delta R$ and $(1\beta)\Delta R\}$, and one $\Sigma[\mu]^{20}_{D\,obs}$, $\{(2\alpha)\Delta R+(4\beta)\Delta R\}$ were shown by Eqs. (4), (8) and (7). These values which were obtained quasi-empirically, seem to be reasonable, from the viewpoint of the geometry of the molecule, as described below :

[1]—The orientation of 1α is essentially perpendicular to the hypothetical flat plane which is symbolized by $(Flar)^m$-plane*13 in Fig. 1 (axial bond), while that of 1β is almost in parallel with this flat plane (equatorial bond)[11]. (ref. Fig. 1). Accurately speaking, the direction of 1β projects out, greatly inclined toward the $(Flar)^1$-plane*14 on the opposite side of 1α. Then, $(1\alpha)\Delta R$ and $(1\beta)\Delta R$ should be opposite in sign, and the absolute value of the former must be far greater than that of the latter*15. These conditions, which are required from the standpoint of the geometry of the molecule, are all satisfied by Eqs. (4) and (8). This indicates that Eqs. (4) and (8) are reasonable in their sign and magnitudes.

[2]—4β and 2α are axial and equatorial orientations respectively. (ref. Fig. 1). Then, the absolute value of $(4\beta)\Delta R$ must be far greater than that of $(2\alpha)\Delta R$. In other words, the sign of $(2\alpha)\Delta R+(4\beta)\Delta R$ should be the same as that of $(4\beta)\Delta R$..... (Condition A)

On the other hand, the positions of C^1- and C^4- atoms are on the opposite half of the aldopyranose-ring together, in relation to the symmetrical plane of this ring in which the centers of the ring-O-atom (represented by O*) and C^3- atom lie. Of course, the orientations of α and β project out to the opposite sides of $(Flar)^m$-plane together; then the second condition is obtained.

$(1\alpha)\Delta R$ and $(4\beta)\Delta R$ must be equal to each other in their sign*15.....(Condition B) Referring to Conditions A and B, it is concluded as below; $\{(2\alpha)\Delta R+(4\beta)\Delta R\}$ and $(1\alpha)\Delta R$ should have the same sign..... (Condition C)

This condition is satisfied by Eqs. (4) and (7). As Eq. (4) has already been proved to be reasonable, above, Eq. (7) is concluded also to be probable.

In spite of the difficulties of calculating them by the purely theoretical procedure, the values of the terms containing R could be thus obtained quasi-empirically.

Now, $[M]^{20}_D(W)$ of three pentoses, can be calculated by using the values $[\mu]^{20}_{D\,obs}$s, above obtained. The $[\mu]^{20}_{D\,obs}$ in the case of β-D-xylose are $(1\beta)\Delta(2\alpha)$, $(1\beta)\Delta(3\beta)$, $(1\beta)\Delta(4\alpha)$, $(1\beta)\Delta R$, $(2\alpha)\Delta(3\beta)$, $(2\alpha)\Delta(4\alpha)$, $(2\alpha)\Delta R$, $(3\beta)\Delta(4\alpha)$, $(3\beta)\Delta R$ and $(4\alpha)\Delta R$*16. Then the calculated value of $[M]^{20}_D(W)$ of β-D-xylose is to be given by the following equation. (ref. Table II and Eqs. (2), (3), (8)).

$$[M]^{20}_D(W)=\Sigma[\mu]^{20}_{D\,obs}=-26.8+0+0+(1\beta)\Delta R+26.8+0+(2\alpha)\Delta R-26.8+(3\beta)\Delta R+(4\alpha)\Delta R=-31.6^{*17}$$

The corresponding observed value of $[M]^{20}_D(W)$ of β-D-xylose has not been found in any referential literatures, but Hudson reported that $[M]^{20}_D$ of this pentose in 80% alcoholic solution is −30°c), which is almost equal to the above calculated value. Next, this calculation-method was

*13 The molecular model of the aldopyranose-ring, used in this article, is one of Sachse strainless ring conformations and is not perfectly flat. (ref. the previous paper (8)). Then it is improper to use this aldopyranose-ring itself as a standard for comparing the orientations of hydroxyl O atom in the molecule. $(Flar)^m$-plane was devised for this reason. It is a perfectly flat plane in which the middle points of C^1—C^2, C^2—C^3, C^3—C^4, C^4—C^5, C^5—O^* and O^*—C^1 bonds lie. (ref. Fig. 1). "Flar" is an abbreviated symbol of "Flat Aldose Ring".

11) D. H. R. Barton, O. Hassel, K. S. Pitzer and V. Prelog, *Nature*, **172**, 1096 (1953).

*14 $(Flar)^1$-plane, on which the centre of C^1 atom lies, is parallel with $(Flar)^m$-plane. (ref. Fig. 1).

*15 Concerning the reason for these deductions from the relative geometry of unit groups, refer to Eq. (1) of the previous paper (10).

*16 Ref. Table 1 in this article.

*17 $[\mu]^{20}_{D\,obs}$s for the anomeric differences (*19) of D-xylose and D-ribose, calculated by using McDonald and Beevers' molecular model, are 168.7 and 171.3, respectively. (Concerning this new molecular model, refer to the next paper, This Bulletin, **30**, 920 (1957). In this case, the presumed value of $[M]^{20}_D(W)$ of β-D-xylose becomes −30.6.

c) C. S. Hudson and E. Yanovsky, *J. Am. Chem. Soc.*, **39**, 1013 (1917).

applied to the cases of α-D- and β-D-riboses*18. 131.3 and −38.4 were obtained as the calculated values of $[M]_D^{20}(W)$ of α-D- and β-D-riboses, respectively. The value observed for β-D-ribose is −34.7[d]. The datum of $[M]_D(W)$ of α-D-ribose has not been found in any referential literatures, but the calculated value (131.3) is greater than that of β-D-ribose (−38.4) by 169.7. The magnitude of this difference is equal to the observed value of the anomeric difference*19 of L-arabinose. This fact is to be expected by Hudson's first isorotation rule*20. Thus, the calculated values of $[M]_D^{20}(W)$s of β-D-xylose, α-D-ribose and β-D-ribose seem to be all probable. This fact means that the principle of optical superposition of Kirkwood's idea exists in the pentose group.

*18 In this case, the conditional equation $(3\alpha)\Delta R=0$ was used in calculation. This was obtained from the symmetry of the Ring.

d) F. P. Phelps, H. S. Isbell and W. W. Pigman, *J. Am. Chem. Soc.*, **56**, 747 (1934).

*19 This is the difference in optical rotation of α and β aldoses. (ref. (8)).

*20 Ref. "Introduction" in this article.

Summary

The method of calculation of optical rotatory power, devised by Kirkwood and improved by the author was applied to the pentose group, and it was concluded that the principle of optical superposition of Kirkwood's idea exists in this group.

The author wishes to express his sincere gratitude to Professor S. Tanaka (Kyoto University) for his valuable advice and kind encouragement throughout the course of this study.

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Molecular Rotations of Glucides in Relation to their Structures. IV. The Internal Conformation of C^6—O Bond in D-Mannopyranose and D-Glucopyranose in their Aqueous Solutions

By Shukichi YAMANA

(Received May 14, 1957)

From the results of X-ray analysis on crystalline methyl-D-mannopyranoside, Cox and Goodwin proposed a presumption on the internal conformation of C^6—O bond in D-mannopyranose in crystalline state[1],*1. Mc Donald and Beevers also determined the same conformation in crystalline D-glucopyranose by X-ray analysis about the crystal of this substance[2]. But, for lack of any suitable method, the internal conformation in the molecules in aqueous solutions has been yet undetermind. In aqueous solution, OH group which combines with C^6 atom is considered to be free to rotate about the axis of C^5—C^6 bond and the position which has minimal potential and makes that OH group rest, will be decided principally by steric repulsions and secondarily by electrostatic

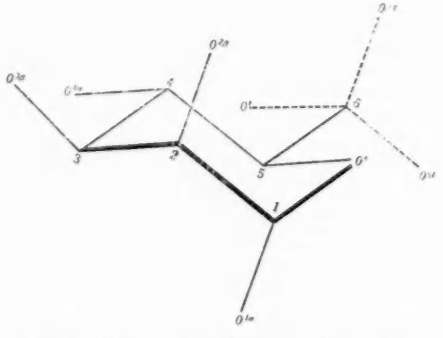

Fig. 1. Perspective drawing of the molecular model of α-D-mannopyranose
gI—*gauche* I (Φ=120°), *gII*—*gauche* II (Φ=240°), *t*—*trans* (Φ=0°)

1) E. G. Cox and T. H. Goodwin, *J. Chem. Soc.*, **1932**, 1844.

*1 Cox and Goodwin presumed that the aldopyranose-ring is flat, but their presumption was proved to be improper by McDonald and Beevers. (ref. (2)).

2) T. R. R. McDonald and C. A. Beevers, *Acta Cryst.*, **5**, 654 (1952).

TABLE I

Name	Unit Groups	$[M]_D^{20}(W)$	Lit.
α-D-mannose	$[(OH)^{1\alpha}, (OH)^{2\beta}, (OH)^{3\beta}, (OH)^{4\alpha}, (CH_2OH)^{5\beta}$, Ring]	53.8°	(a)
β-D-mannose	$[(OH)^{1\beta}, (OH)^{2\beta}, (OH)^{3\beta}, (OH)^{4\alpha}, (CH_2OH)^{5\beta}$, Ring]	−29.3°	(a)
α-D-rhamnose	$[(OH)^{1\alpha}, (OH)^{2\beta}, (OH)^{3\beta}, (OH)^{4\alpha}, (CH_3)^{5\beta}$, Ring]	14.0°	(b)
β-D-rhamnose	$[(OH)^{1\beta}, (OH)^{2\beta}, (OH)^{3\beta}, (OH)^{4\alpha}, (CH_3)^{5\beta}$, Ring]	−63.0°	(b)

forces and hydrogen bonding forces between the atoms in the molecule[3]. (Fig. 1).

According to the idea that the optical rotatory power should be a function of the angle which defines the internal conformation of CH_2Cl group in that molecule, Wood, Fickett and Kirkwood calculated the value of the optical rotatory power of 1,2-dichloropropane, and checked these values comparing them with the observed values. By this method, they could determine the absolute configuration of 1,2-dichloropropane[4]. The author wants to offer a new method of presuming the internal conformation of a bond. The principle of this method is the calculation in reversing order of Kirkwood's method, described above. By using this method, the author was able to presume the internal conformations of C^6—O bond in D-mannopyranose and D-glucopyranose in aqueous solutions.

Molecular Model and Data Used*2

Unit groups in hexose molecules under question and their corresponding molecular rotations, are shown in Table I. (Fig. 1).

In this paper, the hypothetically ideal model of Reeves' C 1 conformation*3 and Mc Donald and Beevers' model*4 which was determined by X-ray analysis of crystalline α-D-glucose, were used as the molecular model. The optical data for OH group and H atom, used in the previous paper[7], were also employed without any correction. The refractive indices of the aqueous solutions of glucides under question are presumed to be nearly equal (1.34)*5.

Method Proposed

The geometry of D-mannopyranose, except that of C^6—O bond, has been determined in the previous paper[6]. At first, the author intended to calculate the optical rotatory power of D-mannopyranose as a function of the angle Φ which defines the internal conformation of C^6—O bond*6 according to Kirkwood's polarizability theory. But, in order to avoid the complexity of calculation, the author used the anomeric difference*7 instead of the molecular rotation itself, and compared the calculated values with the observed one to presume the internal conformation of C^6—O bond. In the molecule of α-D-mannopyranose, $\sum[\mu]^{20}_{D\text{obs}}$, caused by the dynamical coupling effect of $(OH)^1$ is $(1\alpha)\text{Å}(2\beta)$*2 $+(1\alpha)\text{Å}(3\beta)+(1\alpha)\text{Å}(4\alpha)+(1\alpha)\text{Å}(CH_2OH)^{5\beta}+(1\alpha)\text{Å}R$, and the value in the molecule of β-D-mannopyranose is also calculated as

$$(1\beta)\text{Å}(2\beta)+(1\beta)\text{Å}(3\beta)+(1\beta)\text{Å}(4\alpha)+(1\beta)\text{Å}(CH_2OH)^{5\beta}+(1\beta)\text{Å}R^{5,7)}$$

Therefore, $[\mu]^{20}_{D\text{obs}}$ for the anomeric difference*7 of D-mannopyranose should be equal to the difference between the above two $\sum[\mu]^{20}_{D\text{obs}}$s. That is

$$\{(1\alpha)\text{Å}(2\beta)-(1\beta)\text{Å}(2\beta)\}+\{(1\alpha)\text{Å}(3\beta)-(1\beta)\text{Å}(3\beta)\}+\{(1\alpha)\text{Å}(4\alpha)-(1\beta)\text{Å}(4\alpha)\}+\{(1\alpha)\text{Å}(CH_2OH)^{5\beta}-(1\beta)\text{Å}(CH_2OH)^{5\beta}\}+\{(1\alpha)\text{Å}R-(1\beta)\text{Å}R\}\equiv P$$

By the similar treatment, $[\mu]^{20}_{D\text{obs}}$ for the

3) S. Mizushima, Y. Morino and T. Shimanouchi, *J. Chem. Soc. Japan, Pure Chem. Sec.* **73**, 621 (1952); I. Miyagawa, ibid. **75**, 1169 (1954).

4) W. W. Wood, W. Fickett and J. G. Kirkwood, *J. Chem. Phys.*, **20**, 561 (1952).

*2 Abbreviated symbols, used in this paper, are the same as those in the preceding article (5).

5) S. Yamana, This Bulletin, **30**, 916 (1957).

a) C. N. Rüber and J. Minsaas, *Ber.*, **60**, 2402 (1927).

b) C. S. Hudson and E. L. Jackson, *J. Am. Chem. Soc.*, **59**, 1076 (1937).

*3 See the previous paper (6).

6) S. Yamana, This Bulletin, **30**, 207 (1957).

*4 The shape of McDonald and Beevers' model is almost the same as that of the ideal model of Reeves' C 1 conformation, above mentioned. Mc Donald and Beevers decided the following values for the bond lengths and the valency angles in crystalline α-D-glucose molecule[2)]; C^1—C^2 1.54 Å; C^2—C^3 1.56 Å; C^3—C^4 1.53 Å; C^4—C^5 1.54 Å; C^5—O^* 1.40 Å; O^*—C^1 1.42 Å; C^1—O^1 1.32 Å; C^2—O^2 1.41 Å; C^3—O^3 1.44 Å; C^4—O^4 1.40 Å; C^5—C^6 1.53 Å; C^6—O^6 1.32 Å; O^*—C^1—C^2 110°; C^1—C^2—C^3 104°; C^2—C^3—C^4 110°; C^3—C^4—C^5 108°; C^4—C^5—O^* 109 1/2°; C^5—O^*—C^1 111°; O^*—C^1—O^1 112°; C^2—C^1—O^1 115°; C^1—C^2—O^2 109°; C^3—C^2—O^2 109°; C^2—C^3—O^3 102°; C^4—C^3—O^3 108°; C^3—C^4—O^4 110 1/2°; C^5—C^4—O^4 112°; C^4—C^5—C^6 112°; O^*—C^5—C^6 110°; C^5—C^6—O^6 113°.

7) S. Yamana, This Bulletin, **30**, 203 (1957).

*5 ref. *8 in the previous paper (6).

*6 The author defined an angle Φ specifying the internal conformation as follows; (i) Φ is zero at the situation which the O atom in C^6—O bond and the ring-O-atom (represented by O^*) are in *trans* position each other. (ii) Its positive direction of rotation from zero increases the distance between C^4-atom and the O atom in C^6—O bond.

*7 This is the difference in optical rotation of α and β aldoses. (ref. (6)).

anomeric difference of D-rhamnopyranose*8 is obtained as

$$\{(1\alpha)\,\lambda\,(2\beta)-(1\beta)\,\lambda\,(2\beta)\}+\{(1\alpha)\,\lambda\,(3\beta)-(1\beta)\,\lambda\,(3\beta)\}+\{(1\alpha)\,\lambda\,(4\alpha)-(1\beta)\,\lambda\,(4\alpha)\}+\{(1\alpha)\,\lambda\,(CH_3)^{5\beta}-(1\beta)\,\lambda\,(CH_3)^{5\beta}\}+\{(1\alpha)\,\lambda R-(1\beta)\,\lambda R\}\equiv Q$$

Both of the above obtained two $[\mu]^{20}_{D\text{obs}}$s (P and Q) contain the terms concerning the aldopyranose-ring R. It is impossible to calculate these terms at present. Then, in order to avoid this difficulty, Q was subtracted from P and the terms concerning R disappear as below:

Difference between the anomeric difference of D-mannopyranose (P) and that of D-rhamnopyranose (Q) is as follows,

$$P \text{ minus } Q=[(1\alpha)\,\lambda\,(CH_2OH)^{5\beta}-(1\beta)\,\lambda\,(CH_2OH)^{5\beta}]-[(1\alpha)\,\lambda\,(CH_3)^{5\beta}-(1\beta)\,\lambda\,(CH_3)^{5\beta}]$$

The terms of this difference can be simplified by the following procedure. $(CH_2OH)^{5\beta}$ may be considered to occur by replacing an H atom in $(CH_3)^{5\beta}$ by an OH group. Becauses of its small polarizability, $[\mu]^{20}_{D\text{obs}}$, caused by the dynamical coupling effect of H atom, may be neglected[8],*9. Then (P minus Q) becomes as below*10;

$$[(1\alpha)\,\lambda\,\{(CH_2)^{5\beta}+(OH)^6\}-(1\beta)\,\lambda\,\{(CH_2)^{5\beta}+(OH)^6\}]-[(1\alpha)\,\lambda\,\{(CH_2)^{5\beta}+(H)^6\}-(1\beta)\,\lambda\,\{(CH_2)^{5\beta}+(H)^6\}]=(1\alpha)\,\lambda\,(OH)^6-(1\beta)\,\lambda\,(OH)^6$$

Referring to the previous papers[5-7], the value for $\{(1\alpha)\,\lambda\,(OH)^6-(1\beta)\,\lambda\,(OH)^6\}$ was calculated as a function of Φ*11. The results of calculation are given in Table II.

$(53.8+29.3)-(14.0+63.0)=6.1$*12 is gained as the corresponding observed value. This value coincides with the calculated values in Table II when Φ is 0° and 240°*13. Therefore, the internal conformation of C^6—O bond should correspond to one or both of these two angles. According to Mizushima, Morino and Shimanouchi's empirical rule*14, the molecule of XH_2C-CH_2Y type can exist in several forms of rotational isomer in liquid or gaseous state. Then, there may exist an equilibrium between these two types of internal conformations of C^6—O bond in aqueous solutions. These two types happen in the two of the three structures of potentially minimal 1,2-dichloropropane[9] (*trans* form and *gauche* II form). (Fig. 2)*15

TABLE II

$\sum[\mu]^{20}_{D\text{obs}}$ FOR THE DIFFERENCE BETWEEN THE ANOMERIC DIFFERENCE OF D-MANNOPYRANOSE (P) AND THAT OF D-RHAMNOPYRANOSE (Q), AS A FUNCTION OF Φ

Φ	Ideal Model	McDonald and Beevers' Model
0	7.2	7.3
20	2.8	2.8
40	− 3.0	− 3.1
60	−10.4	−10.1
80	−15.6	−16.8
100	−18.9	−21.2
120	−18.2	−21.5
140	−13.8	−17.5
160	− 8.1	−11.0
180	− 2.6	− 4.3
200	1.7	1.3
220	4.7	5.4
240	7.2	8.3
260	9.2	10.3
280	10.8	11.8
300	11.6	12.3
320	11.4	11.9
340	9.9	10.3

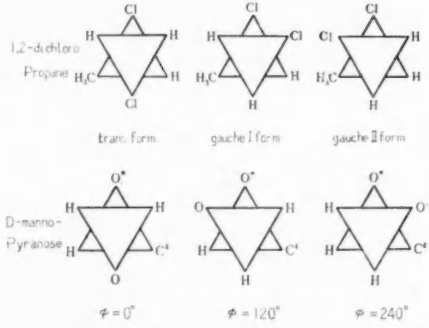

Fig. 2. Internal conformation along the C^6—C^5 axis.

8* D-rhamnopyranose has been confirmed to have Reeves' C 1 conformation by the experiments of cuprammonium-glycoside complexes. (R. E. Reeves, *J. Am Chem. Soc.*, **72**, 1499 (1950).

8) J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).

*9 Consequently. $(1\alpha)\,\lambda\,(H)^6=1\beta\,\lambda\,(H)^6=0$

*10 $(OH)^6$ and $(H)^6$ mean OH group and H atom which combine with the C atoms in $(CH_2OH)^{5\beta}$ and $(CH_3)^{5\beta}$, respectively.

*11 When using McDonald and Beevers' model, κ is $241.2/19.68(n^2+2)$. Then, in order to obtain $[\mu]^{20}_{D\text{obs}}$ from $[\mu]^{20}_{D\text{calcd}}(3/n^2+2)$, the latter must be multiplied by $80.4/19.68=4.085$.

*12 Ref. Table 1 in this paper.

*13 When using the value of C—O bond length of 1.48 Å (M. Kimura, *J. Chem. Soc. Japan, Pure Chem. Sec.* **71**, 18 (1950)), κ is $13.2/n^2+2$ and both of the calculated values of the difference between two anomeric differences, corresponding to $\phi=0°$ and 240°, become 7.5.

*14 Ref. "Discussion on the Results" in this article.

9) I. Miyagawa, *J. Chem. Soc. Japan, Pure Chem. Sec.* **75**, 1165 (1954).

*15 But the O atom in D-mannopyranose molecule corresponds to the Cl atom in 1,2-dichloropropane molecule.

The above obtained two types of internal conformation of C^6—O bond in D-mannopyranose in aqueous solution are presumed to happen also in D-glucopyranose. The reason for this is as follows; If the orientation of $(OH)^{2}$*16 of D-mannopyranose changes from β to α, D-glucopyranose-molecule can be obtained. But the distance between the O atom in $(OH)^2$ and that in C^6—O bond is too large*17 to give rise to any influence of the orientation-change of $(OH)^2$ on the internal conformation of C^6—O bond*18.

Discussion on the Results

According to Mc Donald and Beevers' experiment[2], the internal conformation of C^6—O bond in α-D-glucopyranose, in crystalline state, is almost consistent with the conformation corresponding to *gauche* 1 form*19. Then, it may be concluded that, when α-D-glucopyranose is dissolved in water, the internal conformation of C^6—O bond changes from *gauche* 1 to *trans* and *gauche* II types. On the other hand, Mizushima, Morino and Shimanouchi[3] found an empirical rule that the molecule of XH_2C—CH_2Y type, may be able to exist in several forms of rotational isomer in liquid or gaseous state, and one of these forms is identical with the form in the solid state. The author's conclusion*20 on the conformation of C^6—O bond in α-D-glucose somewhat seems to deviate from this empirical rule. This disharmony may be explained as follows. As Mc Donald and Beevers observed[2] that O^6- atom of a molecule of crystalline α-D-glucopyranose is combined, by hydrogen bonding forces, with both O^2- and O^3- atoms of the neighboring molecule*21, the internal conformation of C^6—O bond in crystalline α-D-glucopyranose is greatly influenced by the atoms of the other molecules in the crystal. These forces contribute to make O^6- atom rest at such a position that C^6—O bond takes *gauche* I conformation. But when α-D-glucose is dissolved in water, each molecule is separated from other molecules and the influences of the neighboring molecule decrease. Therefore, the internal conformation of C^6—O bond in that molecule is influenced principally by the atoms in its own molecule. Moreover, as the distance between $O^{4\alpha}$ and O^6 is almost equal to that between O^6- and C^4- or O*-atoms (ref. Table III), the influence of $O^{4\alpha}$ on O^6 may be unable to neglect. (Fig. 1)

TABLE III

THE DISTANCES BETWEEN O^6- ATOM AND ITS NEIGHBORING ATOMS IN MCDONALD AND EEEVERS' MODEL AND THE IDEAL MODEL*22

	trans	*gauche* I	*gauche* II
O^6 and $O^{4\alpha}$	2.69Å(2.51)	4.18Å(4.19)	3.45Å(3.42)
O^6 and C^4	2.96Å(2.89)	3.73Å(3.76)	2.96Å(2.89)
O^6 and O*	3.57Å(3.76)	2.81Å(2.89)	2.81Å(2.89)

In Table III, the distance between O^6 and $O^{4\alpha}$ in *trans* form is the shortest (this is 2.69 Å) and is just equal to the length of a hydrogen bond*23. Therefore, O^6- atom which is only one movable atom in Table III, may be attracted by $O^{4\alpha}$- atom most strongly and is forced to rest at the *trans* position*24 by making a hydrogen bond between $(OH)^6$ and $(OH)^{4\alpha}$ group. In order to find a reason for non-existence of *gauche* I form in aqueous solutions against its expectation of appearance by the empirical rule, the affinity of O^6- atom to *gauche* I and *gauche* II positions should be compared. By using Table III, it is noticed that the distances between O^6 and any one of these neighboring atoms ($O^{4\alpha}$, C^4 and O*) in *gauche* I form is always larger than (or at least equal to) that in *gauche* II form, respectively. Then the total of the attractive forces of the neighboring atoms on O^6-atom in *gauche* II form should be greater than that in *gauche* I form. This fact causes the affinity of O^6- atom to *gauche* II rather than to *gauche* I position. These may be the reason for non-existence of *gauche* I form of rotational isomer of D-glucopyranose and D-mannopyranose in aqueous solutions.

*16 $(OH)^2$ means OH group which combines with the C^2- atom of the aldopyranose-ring.

*17 The distances between the O atom in $(OH)^2$ and that in C^6—O bond are as below;

	longest	shortest
$(OH)^{2\alpha}$	6.49 Å (at Φ=40°)	6.03 Å (at Φ=220°)
$(OH)^{2\beta}$	5.82 Å (at Φ=60°)	4.56 Å (at Φ=240°)

*18 It must be remembered here, that the coefficient 4.123 (in the previous paper (5)) and 4.085 (in *11 of this article), used in calculating $[\mu]^{20}_{Dobs}$ from $[\mu]^{20}_{Dcalcd}$ $(3/n^2+2)$ were obtained under this hypothesis.

*19 The observed internal conformation of C^6—O bond corresponds to Φ=111½° in Mc Donald and Beevers' model.

*20 The possible internal conformation of C^6—O bond are *trans* and *gauche* II in aqueous solution but *gauche* 1 only, in crystalline state.

*21 O^2—O^6 2.74 Å; O^3—O^6 2.70 Å

*22 The values in the ideal model are given in parentheses.

*23 cf. *21 in this paper.

*24 This is the position of O^6- atom which corresponds to *trans*-type conformation of C^6—O bond.

Summary

The internal conformation of C^6—O bond in the molecules of D-mannopyranose and D-glucopyranose in aqueous solutions were presumed by a new method, and was concluded to take *trans* (mostly) and *gauche* II (only sometimes) forms.

The author wishes to express his sincere gratitude to Professor S. Tanaka (Kyoto University) for his valuable advice and cordial encouragement throughout the course of this study. Also the author wants to express his great thanks to Assistant Professor Y. Saito (Osaka City University) for his kind teaching of the fundamental knowledge in X-ray analysis.

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Absorption Spectra and Chemical Behavior of the Hexahydroxylamine Cobalt(III) Complex[1]

By Hayami YONEDA

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In general, complexes of a transition metal ion having an unsaturated *d*-shell have several absorption bands in the region ranging from the near infrared to the ultraviolet. These bands can be considered, to a good approximation, as arising from the energy transitions between the *d*-levels which are split by the electrical field set up by the ligands[2]. The energy transitions between such split *d*-levels correspond to the frequencies of the absorption maxima of the complex. As the energy intervals of such split *d*-levels increase with the increase of the electrical field by the ligands, the strength of the electrical field produced by various ligands can be estimated by comparing the maximum position of the absorption band of various complexes. Such a comparison must have a special significance in the case of complexes of the same type having a series of ligands which have the same donor atom. Because, by such an investigation, we can look for the main factors governing the stability of the coordination bond. With this purpose, the absorption spectra of a series of hexammine nickel(II) and tetrammine platinum (II) complexes were investigated in the previous study[3]. As a result, it was found that the weaker the basic strength of an amine, the shorter the wavelength at which the characteristic absorption bands of the complex appear. This fact indicates that the interaction between the metal ion and the ligands increases with the decreasing order of the basic strengths of co-ordinating amines. A similar rule must be observed more clearly in the case of cobalt(III) ammine complex which is regarded as having more covalent character. With this in mind, the absorption spectrum of hexahydroxylamine cobalt(III) complex was measured and compared with the hitherto measured data of other hexammine cobalt(III) complexes. The result indicated that both the first and the second absorption band of the hydroxylamine complex lay in a much shorter wavelength than those of other hexammine complexes. As the basic strength of hydroxylamine is much weaker than that of ammonia, methylamine or ethylenediamine, this result indicates that the same rule as found in nickel(II) and platinum(II) ammine complexes holds also in the cobalt(III) complex.

In the course of this study it was found that hexahydroxylamine cobalt(III) complex was very unstable in a solution and decomposed gradually to the cobalt(II) salt. This fact seemed to be apparently inconsistent with the above mentioned spectrochemical result. To clarify the cause of this inconsistency, a detailed study of the decomposition of the complex was carried out by measuring the rate of

1) This paper is Part II of "Stabilities of Metal Ammine Complexes".
2) For examle, L. E. Orgel, *J. Chem. Phys.*, **23**, 1004 (1955).
3) Yoneda, This Bulletin, **30**, 132 (1957).

decomposition under various conditions. It was revealed that a very strong binding between the central cobalt(III) ion and the nitrogen atom of the co-ordinating hydroxylamine caused the instability of the complex as a whole. Accordingly, this proved that the instability of hexahydroxylamine cobalt(III) complex supported the validity of the above mentioned rule.

Experimental

Preparation of Hexahydroxylamine cobalt (III) Complex.—Hexahydroxylamine cobalt(III) chloride $[Co(NH_2OH)_6]Cl_3$ was prepared by the following modification of Werner's method[4]. Three g. of *trans*-dichloro-diethylenediamine-cobalt(III) chloride $[Co\ en_2\ Cl_2]Cl$ and 4 g. hydroxylamine hydrochloride $NH_2OH \cdot HCl$ were dissolved in a small amount of water. The solution was cooled below 0°C. To this solution, a concentrated solution containing 2 g. sodium hydroxide was added, and the mixed solution was vigorously stirred. After a while, a light brown precipitate appeared. Then a large excess of concentrated hydrochloric acid was added until the color of the solution became dark green. Within a few minutes, the desired complex began to appear in the form of glittering golden leaflets. After kept standing for an hour, the crystals were filtered and redissolved in slightly acid warm water (near above 50°C in temperature) and reprecipitated by the addition of concentrated hydrochloric acid. The corresponding bromide and perchlorate of the complex* were obtained by the addition of the concentrated corresponding acid to the concentrated solution of the chloride.

Measurement of the Absorption Spectra.—When hexahydroxylamine cobalt(III) complex is dissolved in distilled water, it decomposes fairly rapidly. This decomposition can be stopped by the addition of strong acids. Therfore, the absorption spectrum of the complex was measured in 0.1N perchloric acid solution. In order to know how the absorption spectrum changes with the variation of pH, the absorption spectrum was measured in a buffer solution of pH 4.8. The absorption measurement was carried out using Beckman DU spectrophotometer.

Determination of the Rate of Decomposition.—The rate of decomposition of hexahydroxylamine cobalt(III) complex was measured in the following buffer solutions;

Mixture of 0.5 N acetic acid and 0.5 N sodium acetate pH 4.8

Mixture of 0.05 N acetic acid and 0.5 N sodium acetate pH 5.8

The buffer solution was heated to the desired temperature, and in this solution the complex was dissolved quickly, to let the decomposition start. Five cc. of the reacting solution was taken out with a pipette and pourd into 2 cc. of 2 N perchloric acid, to stop the decomposition. At appropriate time-intervals, this procedure was repeated. Thus, we obtained a series of solutions which contained the complex in various concentrations. Therefore, if we can determine the concentration of the complex in these solutions, we can trace the decomposition of the complex. For this purpose, the optical densities of these solutions were measured at several wavelengths near the maximum position of the first absorption band of the complex. The ratios of the optical densities of these solutions to that of the innitial solution give the relative concentrations of the complex. From these values we can trace the decrease in the concentration of the complex in the decomposition process.

4) A. Werner und E. Berl, Ber., **38**, 893 (1905).

* The crystals of bromide and chloride cannot be preserved for a long time, because they change gradually into the cobaltous salt even in a closed vessel. On the contrary, perchlorate does not change even in summer or in the sunlight, in the absence of moisture.

Results

The results of the absorption measurement are shown in Fig. 1 and Table I. In the Table are listed also the maximum positions of the first and the second absorption band of other hexammine cobalt (III) complexes hitherto measured.

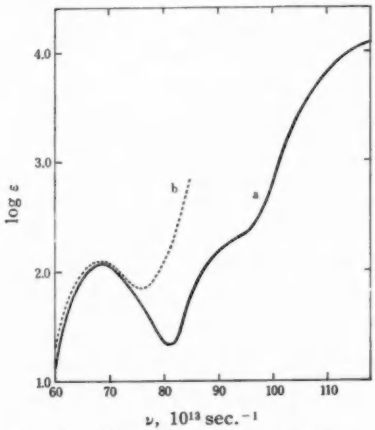

Fig. 1. The absorption spectra of $[Co(NH_2OH)_6](ClO_4)_3$.
a) in 0.1 N $HClO_4$.
b) in the buffer solution of pH 4.8.

The results of the measurement of the rate of decomposition of the complex are represented in Figs. 2 and 3. Each curve in these figures can be transformed into a straight line, when the logarithmic value of the concentration is taken as an ordinate and the time as an abscissa. This indicates that the decomposition of the complex proceeds apparently in the form of the first order reaction. The rate constants of the reaction are as follows: in the solution of pH 4.8 at 50°C 0.0990min.

TABLE I

MAXIMUM POSITIONS OF THE FIRST AND THE SECOND ABSORPTION BAND OF COBALT(III) HEXAMINE COMPLEXES (in 10^{13} sec.$^{-1}$ unit)

Complex	pK of the co-ordinating amine	1st Band	2nd Band
$[Co(NH_2OH)_6]^{3+}$	6.03[5]	68.5 (log ε 2.07)	ca. 94 (log ε ca. 2.3)
$[Co\ en_3]^{3+}$	8.55*[6]	63.9	88.4
$[Co(NH_3)_6]^{3+}$	9.25[7]	62.9	88.3
$[Co(NH_3)_4(CH_3NH_2)_2]^{3+}$	10.63**[7]	61.9	86.3

* mean value of pK_1 7.19 and pK_2 9.92

** pK_a of CH_3NH_2

liter mole., 45°C 0.0462 and 40°C 0.0198; in the solution of pH 5.8 at 35°C 0.0815 min. liter mole., 30°C 0.0347 and 25°C 0.0139.

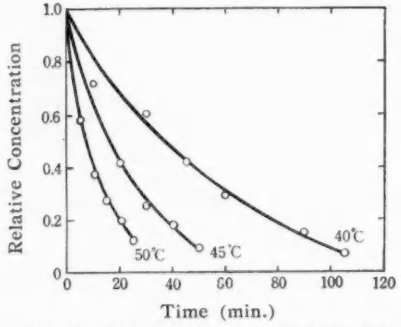

Fig. 2. Decomposition of $[Co(NH_2OH)_6](ClO_4)_3$ in the buffer solution of pH 4.8.

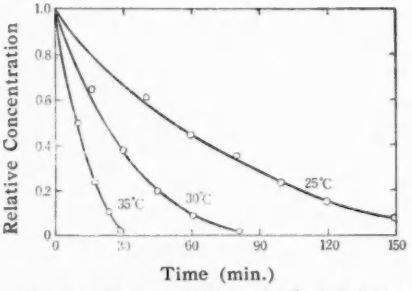

Fig. 3. Decomposition of $[Co(NH_2OH)_6](ClO_4)_3$ in the buffer solution of pH 5.8.

Taking the logarithmic values of these rate constants as an ordinate, and the reciprocals of the absolute temperatures as an abscissa, a straight line is obtained. From its slope, the activation energy is calculated to be 32.5 kcal mole^{-1}. As the two straight lines in Fig. 4 are parallel it is concluded that the activation energy is independent of the variation of pH. Besides, as the ordinate-interval between the two lines is unity, it is concluded that the reaction rate is proportional to the concentration of the hydroxyl ion.

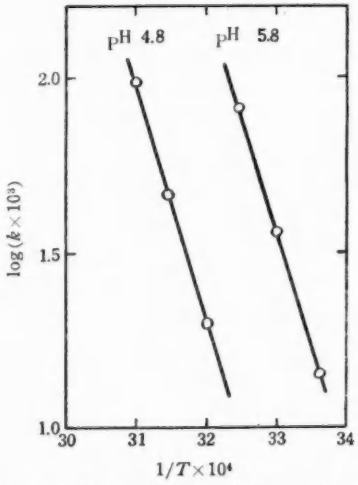

Fig. 4. Determination of the activation energy for the decomposition of $[Co(NH_2OH)_6](ClO_4)_3$ from plots of $\log k$ against $1/T°$.
a) $\log k$ for pH 4.8.
b) $\log k$ for pH 5.8.

Discussion

As expected, the first and the second absorption bands of hydroxylamine cobalt (III) complex lie much farther in the shorter wavelength than those of other hexammine cobalt(III) complexes hitherto measured. In addition, as is easily seen in Table I, both the first and the second absorption band of hexammine cobalt(III) complexes show the blue shift with the increase in the acid dissociation constants

5) H. Hagisawa, Bull. Inst. Phys. Chem. Research, Tokyo, **20**, 251 (1954).
6) G. A. Carlson, J. P. McReynolds and F. H. Verhoek, *J. Am. Chem. Soc.*, **67**, 1334 (1945).
7) H. S. Harned and B. B. Owen, ibid. **52**, 5079 (1930).
8) Y. Shimura and R. Tsuchida, This Bulletin, **29**, 311 (1956).
9) J. Brigando, *Compt. rend.*, **236**, 708 (1953).

of the co-ordinating amine. This fact indicates that the weaker the basic strength the co-ordinating amine has, the more strongly it combines with the central cobalt(III) ion. This tendency can be concluded also from the marked red shift of the charge transfer band of hexahydroxylamine cobalt(III) complex compared with that of hexa-ammonia cobalt(III) complex. In the free state, both hydroxylamine and ammonia have the absorption band in the shorter wavelength region near 2000 Å. This absorption band of hydroxylamine shows a marked red shift when it is co-ordianted to the cobalt(III) ion and this band is superposed partly upon the second absorption band of the complex. In contrast to this, the absorption band of ammonia shows only a slight red shift in the co-ordinated state. Here, it is noteworthy that in the case of the hydroxylamine complex the red shift of such a charge transfer band becomes much more marked as the pH of the solution increases, and conceals completely the second absorption band. This indicates that the interaction between the central cobalt(III) ion and the co-ordinating hydroxylamine increases when the pH of the solution increases. Such a marked change of the charge transfer band was not observed in the case of the ammonia complex, so far as the perchlorate of the complex was concerned. Such a strong interaction between hydroxylamine and the cobalt(III) ion was presumed from the method of preparation of hexahydroxylamine cobalt(III) chloride. As described in the experimental part, hydroxylamine can expel even ethylenediamine from its co-ordination sphere, which is very firmly linked to the central cobalt(III) ion.

When the electronic cloud of the nitrogen atom of hydroxylamine penetrates so deeply into the central cobalt(III) ion, we can expect the relaxation of the N–H bond, which facilitates the proton dissociation in an aqueous solution. Therefore, the following equilibrium may be formed;

$$[Co(NH_2OH)_6]^{3+} + H_2O \rightleftarrows [Co(NH_2OH)_5NHOH]^{2+} + H_3O^+$$

$[Co(NH_2OH)_5NHOH]^{2+}$ thus produced is regarded as the intermediate toward the reduction of the complex. The main reason why hydroxylamine having a strong reducing power does not reduce the cobalt (III) ion and can coexist with the cobalt (III) ion within the hexahydroxylamine cobalt(III) ion, can be attributable to the fact that six co-ordinating hydroxylamine molecules contend with each other and cancel the polarizing power of the cobalt (III) ion. When this balance is lost by the proton dissociation from one co-ordinating hydroxylamine, other hydroxylamine molecules are subjected to the increased polarizing power of the central cobalt(III) ion, so that an electron is easily snatched up by the central cobalt (III) ion, resulting in complete reduction. This is based upon the following empirical rule: the electronic cloud of the ligand, which has lost a proton, must penetrate into the central cobalt(III) ion much more weakly than that of the ligand which keeps a proton. This means that the $NHOH^-$ group is linked to the cobalt(III) ion less covalently and less firmly than the hydroxylamine molecule. As is seen from its position in the spectrochemical series, water is linked to the cobalt(III) ion much more strongly than the hydroxyl ion. A similar relation may be expected in the case of hydroxylamine and $NHOH^-$ too. In fact, the first absorption band of hexahydroxylamine cobalt(III) complex ion makes a slight red shift, when the pH of the solution increases. As the percentage of the complex which has lost a proton must increase more in a weakly acid solution than in a strongly acid solution, and as the red shift means that the field set up by the ligands decreases, the above mentioned red shift suggests that the $NHOH^-$ group is linked to the cobalt (III) ion less firmly than the hydroxylamine molecule. Thus, when one co-ordinating hydroxylamine becomes a less polarizable $NHOH^-$, the degree of the charge neutralization of the central cobalt (III) ion by the co-ordinating electrons of the donor atoms decreases, and consequently, the remaining hydroxylamine molecules in the complex radical is polarized more strongly by the central cobalt (III) ion. Such an increase in polarization induces the reduction. Therfore, if we could depress the proton dissociation by some means, the decomposition of the complex would not take place. Indeed, in a strongly acid solution the decomposition of the complex does not occur. This is the present author's interpretation. With this view, we can understand the cause why penta-, tetra-, and tri-hydroxylamine cobalt(III) complexes such as $[Co(NH_2OH)_5Cl]^{2+}$, $[Co(NH_2OH)_4CO_3]^+$, and

$[Co(NH_2OH)_3(H_2O)_3]^{3+}$ can not be obtained. Even if such complxes could be prepared, the large polarizing power of the central cobalt(III) ion would polarize hydroxylamine over its limit, and the reduction of cobalt(III) to cobalt(II) would take place. Accordingly, if we chose the ligand which decreases the polarizing power of the central cobalt(III) ion very much, we could obtain the cobalt(III) complex containing hydroxylamine. As one of such ligands we know the dimethylglyoximato group*.

The above mentioned view was confirmed further by the investigation of the decomposition of the hexahydroxylamine cobalt (III) complex in an aqueous solution. The result shows that rate of decomposition of the complex is proportional to the concentration of the hydroxyl ion as well as to the concentration of the complex ion. This fact indicates that although the decomposition of the complex is apparently a reaction of the first order in the complex ion, in fact, the rate-determining step of the decomposition is second order in the hydroxyl ion as well as in the complex ion. As such a reaction of the second order in the hydroxyl ion and the complex ion, we can assume the substitution reaction as well as the proton dissociation, such as

substitution

$$[Co(NH_2OH)_6]^{3+} + OH^- \rightarrow [Co(NH_2OH)_5OH]^{2+} + NH_2OH$$

proton dissociation

$$[Co(NH_2OH)_6]^{3+} + OH^- \rightarrow [Co(NH_2OH)_5NHOH]^{2+} + H_2O$$

But as is already stated, the spectrochemical consideration reveals that hydroxylamine is linked to the cobalt(III) ion more firmly than ammonia and that the hydroxyl ion is linked to the cobalt (III) ion less firmly than ammonia. Accordingly, the possibility of substituting the hydroxylamine can be excluded, and the proton dissociation can be regarded as the only possible rate-determining step of the decomposition.

* According to Nakahara's report, the dimethylglyoximato group is situated ahead of ethylenediamine in the spectrochemical series, and forms a strongly covalent bond with the central cobalt(III) ion. A. Nakahara, This Bulletin, **27**, 260 (1954).

Summary

The absorption spectrum of hexahydroxylamine cobalt(III) complex was measured and compared with the hitherto obtained data of the other hexammine cobalt(III) complexes. The frequencies of the first and second absorption bands of this complex were 68.5×10^{13} and ca. 94×10^{13} sec.$^{-1}$ respectively. These values are much greater than those found in other hexammine cobalt(III) complexes, and this fact may indicate the strong interaction between the central cobalt(III) ion and nitrogen atoms of the co-ordinating hydroxylamine. In addition to the study of absorption spectra, the rate of decomposition of this complex was measured. The rate of decomposition was proportional to the concentration of the hydroxyl ion, and the activation energy was 32.5 kcal. mole^{-1}. In order to explain the above results, the following equilibrium, assuming the proton dissociation, was proposed;

$$[Co(NH_2OH)_6]^{3+} + H_2O \rightleftarrows [Co(NH_2OH)_5NHOH]^{2+} + H^3O^+$$

The author wishes to express his sincere thanks to Professor R. Tsuchida of Osaka University for his kind advice and encouragement during this work. The author's appreciation is also expressed to Professor O. Nagai of Wakayama Medical College, who gave the author a chance to use a Beckman DU spectrophotometer, to Mr. S. Kida for his valuable discussion, and to Mr. Y. Morimoto for his experimental help.

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Crystalline Cypridina Luciferin

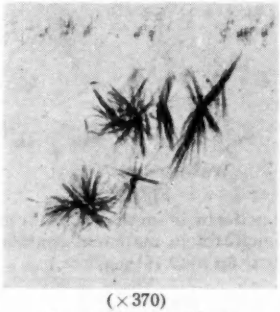
By Osamu SHIMOMURA, Toshio GOTO and Yoshimasa HIRATA

(Received June 3, 1957)

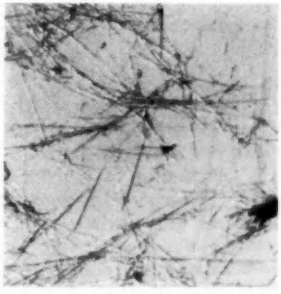
The luminescence of *Cypridina hilgendorfii* has been investigated so far by many workers[1], including Anderson, Kanda and Harvey, and it has been found that firefly luminescence requires ATP and magnesium ion as well as luciferin, luciferase and oxygen for its functioning, whereas *Cypridina* requires only the last three. *Cypridina* luciferin is therefore the simplest system and is most suited for investigating the phenomenon of biological luminescence. For the study of the chemical constitution of *Cypridina* luciferin, it is necessary to obtain pure luciferin. Among several studies concerning the purification of luciferin, the most valuable one is Anderson's benzoylation method[2] reported about twenty years ago. Recently various purification methods have been attempted by Mason[3] and by Tsuji[4]; however, luciferin has as yet not been obtained crystalline. Nevertheless many views relating to the constitution of luciferin have been submitted, including proteose, phospholipid, polyhydroxy benzene, hydroquinones, flavoprotein and chromopeptide[5]. Recently, Mason[7] and Tsuji[5] found that the acid hydrolysis of luciferin afforded many kinds of amino acids.

We have carried out the purification of luciferin and have succeeded in obtaining it crystalline. Investigations on the constitution of luciferin using the crystalline luciferin has also been carried out and preliminary results are reported herewith.

Isolation and crystallization of luciferin. — Pulverized dry *Cypridina hilgendorfii* was de-fatted with benzene and extracted with methanol in hydrogen atmosphere. The extract was purified according to a modified Anderson's benzoylation method[2] and by means of partition chromatography on cellulose powder to obtain "purified" luciferin. Acidification of the purified luciferin solution with hydrochloric acid afforded crystalline luciferin as orange-red needles (Fig. 1).



(×370)
Crystalline Luciferin



(×280)
Oxyluciferin A
Fig. 1

Crystalline luciferin melted at 182—195° (darkened at 175°) it is easily soluble in methanol and ethanol, soluble with difficulty in water and acetone, and insoluble in ether and benzene. The luminescent activity induced by luciferase is 37,000 times as intense as that of the starting material, dry *Cypridina hilgendorfii*. Ninhydrin- and *p*-dimethylaminobenzaldehyde-test were negative. When heated with lime, it decomposed with a skatole-like odor, and the molybdenum blue reaction of its residue was negative. Accordingly, phosphoric acid is probably

1) E. N. Harvey: "Bioluminescence", Academic Press Inc. Publishers, New York, 299 (1952).
2) R. S. Anderson; *J. Gen. Physiol.*, **19**, 301 (1935).
3) H. S. Mason and E. F. Davis; *J. Biol. Chem.*, **197**, 41 (1952).
4) F. 1. Tsuji; *Arch. Biochem. and Biophys.*, **59**, 452 (1955).
5) F. I. Tsuji, A. M. Chase, E. N. Harvey; F. H. Johnson: "The Luminescence of Biological Systems", American Association for the Advancement of Science, p. 127—156 (1955).

absent in luciferin. Ultra-violet and visible spectra of crystalline luciferin were nearly identical with those measured by Chase[6] and Tsuji[5] but the absorption at 435 mμ of the present sample was much stronger (Fig. 2).

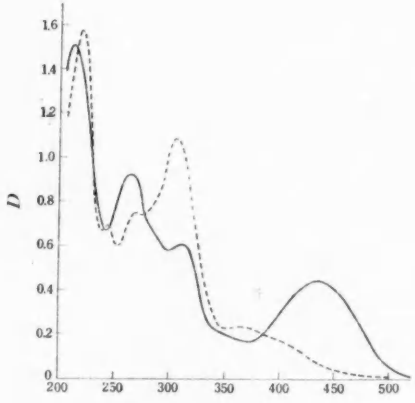


Fig. 2

—— Luciferin in methanol (0.161 mg/5 cc.)
---- Luciferin in methanol containing HCl (ca. 0.1 N) (0.161 mg/5 cc.)

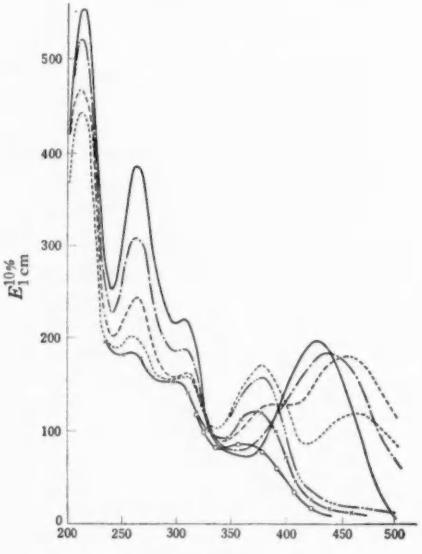


Fig. 3. Aq. luciferin solution (pH 5.6 phosphate buffer).

——— freshly dissolved
— - — after 7 hrs.
------ after 1 day
-------- after 3 days
——— after 7 days
—×— after 12 days
—○— after 52 days

Oxidation of luciferin.—When an aqueous solution of luciferin was allowed to stand in the air, its color changed gradually from yellow to red, and finally became almost colorless. The change of its UV absorption spectra is shown in Fig. 3. After being allowed to stand for 2 days, an ethanolic solution of luciferin afforded three spots as revealed by means of paper chromatography, i. e., one spot was a red colored substance and the other two were colorless blue-fluorescent substances (they were named oxyluciferin A and B) (solvent: ethyl acetate : ethanol : water, 5 : 2 : 3, Rf 0.97, 0.9 and 0.7, respectively). These substances were separated by the method of cellulose powder partition chromatography. The aqueous solutions of these substances were allowed to stand for a few days. Though the solutions of A and B were almost unchanged, the solution of the red substance was decolorized and afforded oxyluciferin A and B. These experiments and the UV absorption spectra suggest that luciferin is oxidized by at least three steps in the air ; i. e., luciferin is first oxidized to the red substance ($\lambda_{max.}$ ca. 465 mμ), subsequently to oxyluciferin A and B ($\lambda_{max.}$ ca. 380 mμ in Fig. 2), and finally decomposed to unknown substances which show no fluorescence and no definite ultraviolet absorption except a maximum at 210 mμ.

The red substance has no luminescence activity, and is decolorized by addition of sodium borohydride. Its color is changed to a bluish violet by addition of concentrated sodium hydroxide solution. The red substance can be obtained easily by oxidation of luciferin with potassium ferricyanide (Fig. 4), though other oxidants oxidize luciferin to colorless substances directly.

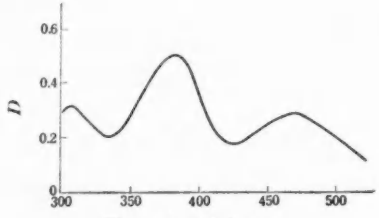


Fig. 4.

6) A. M. Chase and E. H. Brigham, *J. Biol. Chem.*, **190**, 529 (1951).
7) H. S. Mason, *J. Am. Chem. Soc.*, **74**, 4727 (1952).

Oxyluciferin A and B can be obtained in a good yield by addition of ammonia to the luciferin solution. Oxyluciferin A is obtained as yellow needles and melts at 140—148° (darkens at 135°) (Fig. 1).

Oxyluciferin B could not be obtained crystalline and became dark red upon addition of concentrated hydrochloric acid. Oxyluciferin A and B may be very similar substances, because their UV absorption spectra (Fig. 5) and their hydrolysis products (mentioned below) are very similar.

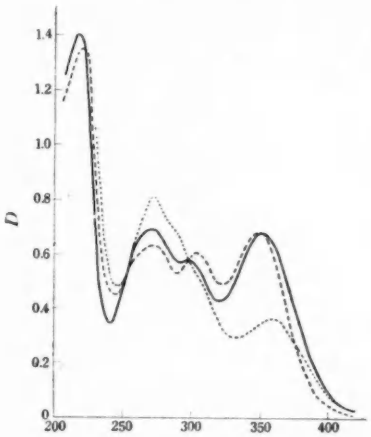


Fig. 5

—— A in phosphate buffer (pH 5.6) 0.084 mg/3 cc.
---- A in MeOH 0.123 mg./3 cc.
..... B in MeOH concn. unknown.

The oxidative decomposition of an aqueous solution of luciferin by air is also influenced by the pH of the solution. Generally, in an acidic medium the oxidation rate is slow and the red substance is stable for a long time, but in an alkaline medium the rate is very great and a yellowish green fluorescent substance is produced as well as A and B. When luciferin was decomposed by luciferase, luminescence occurred and the reaction mixture consisted mainly of oxyluciferin A and a small amount of B.

Hydrogenation of luciferin.—Hydrogenation of luciferin in methanol with Adams' platinum oxide catalyst afforded a colorless substance, the ultraviolet absorption spectrum of which was very similar to that of tryptophan or tryptamine (Fig. 6). The difference in the log D values at 220 and 280 mμ is 0.755, and this value is almost equal to that of tryptophan (0.79) or tryptamine (0.70). It is, therefore, apparent that these two absorption maxima originate in an indole type chromophore and are not caused by overlapping absorptions. Moreover the absorption intensity of hydroluciferin at 220 mμ is stronger than that of luciferin before hydrogenation. These observations suggest that the indole

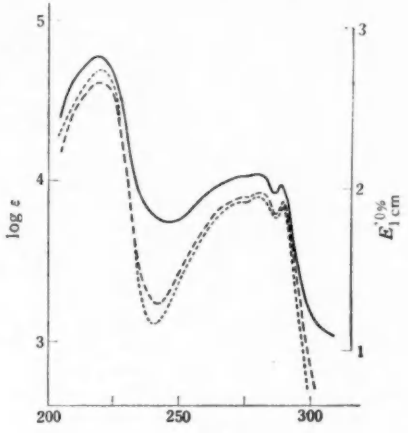


Fig. 6.

—— Hydroluciferin (right scale)
---- Tryptamine } (left scale)
..... Tryptophan }
in methanol

structure in hydroluciferin does not exist in luciferin, but is produced during hydrogenation. Hydrogenation of oxyluciferin A did not afford a substance which has an indole type absorption. The portion which is responsible for the indole type absorption after hydrogenation may be destroyed during oxidation of luciferin to oxyluciferin A. This observation is consistent with the fact that luciferin, and oxyluciferin A and B do not give positive reactions with p-dimethylaminobenzaldehyde (indole test), whereas hydroluciferin affords a positive reaction.

Hydrolysis of luciferin.—Acid hydrolysis of luciferin afforded glycine, isoleucine and a fluorescent substance. Hydrolysis of luciferin with barium hydroxide afforded glycine, isoleucine, a strong ninhydrin positive substance, a fairly large amount of anthranilic acid, and a small amount of indole or indole acetic acid, but no tryptophan. Since tryptophan is readily subject to oxidation, it was considered that tryptophan was decomposed during hydrolysis. Accordingly, the alkali hydrolysis was carried out in the presence

of small amounts of cystein as an antioxidant. Though tryptophan was not obtained either a new ninhydrin positive spot was obtained and identified to be tryptamine; in this case the amount of anthranilic acid was decreased.

Discussion

Acid hydrolysis of crystalline luciferin afforded only two amino acids, glycine and isoleucine; this reult is very different from that of Mason[7] and Tsuji[5], who obtained the following amino acids from the acid hydrolysate of "purified" luciferin: (Tsuji) arginine, leucine, isoleucine, glutamic acid, alanine, valine, sarcosine, lysine, taurine and methionine, and either aspartic acid, threonine, or serine; (Mason) taurine, aspartic acid, threonine, serine, sarcosine, glutamic acid, proline glysine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine. Acid hydrolysis of the "purified" luciferin obtained by the present authors also afforded at least eight amino acids and a fluorescent substance. Since crystalline luciferin and "purified" luciferin have the samc R_f values on paper chromatography in hydrogen atmosphere, it is assumed that the extra amino acids in the hydrolysate of "purified" luciferin are impurities, which may be contained in luciferin as polypeptides. Since glycine is often produced from other substances by decomposition, care should be taken in order to identify it as one of the constituents, but by means of semi-quantitative paper chromatography it was shown that the amount of glycine was almost the sams as that of isoleucine. Thus glycine is regarded to be a component of luciferin.

The comparison of th UV absorption spectra and of the alkali hydrolysates of various indole derivatives (2-methyl indole, 3-methyl indole, 2,3-dimethyl indole, tetrahydrocarbazole, tryptophan, tryptamine, indole acetic acid, indole, etc.) suggest that anthranilic acid and indole derivatives resulting from hydrolysis of luciferin with barium hydroxide were produced from a β-substituted indole type moiety in luciferin.

The facts that hydrogenation of luciferin afforded indole type absorption and that hydrogenation of oxyluciferin A did not, suggest that the indole-type structure may be connected with the luminescent activity of luciferin. Comparison of ε values at 280 mμ of hydroluciferin and tryptophan or tryptamine leads to the result that the mole equivalent of luciferin for one indole group is ca. 500—800.

Experimental

Collected *Cypridina* was dried by being exposed to sunlight and then stored in a vacuum desiccator. Five hundred grams of completely dried *Cypridina* was pulverised and packed in the extraction apparatus (Fig. 7). It was defatted by

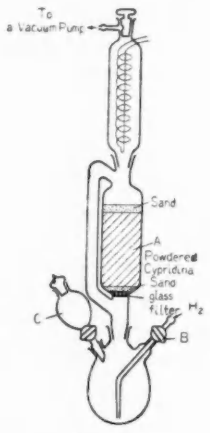


Fig. 7.

refluxing with 1.2 l. of well-dried benzene under reduced pressure (ca. 80 mm.Hg.) for 50 hr. The benzene extract was discarded, wet *Cypridina* was dried in vacuo (about 12 hr.), and then the apparatus was filled with purified hydrogen. The purified hydrogen was obtained by passing hydrogen through a soda-lime tube, red-heated metallic copper packed in a silica tube, and a washing bottle containing concentrated sulfuric acid. Absolute methanol (1.2 l.) was introduced into flask A through funnel C, and the extraction was started under reduced pressure (ca. 80 mm.Hg of hydrogen). After 50 hr. methanol was evaporated in vacuo to give a reddish brown foamy paste. The extraction apparatus was filled again with hydrogen, and 0.5 N hydrochloric acid (850 ml.) was added to the residual paste through funnel C. After being heated at 100°C on a water bath for 10 min. in hydrogen atmosphere, the yellow solution was cooled to about 0° and extracted with ether, at first with 200 ml. and subsequently thrice with 100 ml. The ether extract was discarded. Then the acid solution was extracted several times with *n*-butanol (total ca. 350 ml.), and the butanol extract was purified by a modified Anderson's benzoylation method as follows.

The butanol extract was benzoylated with benzoyl chloride (3 ml. of benzoyl chloride per 100 ml. of butanol soln.) at 0°C. After ten min. the reaction mixture was washed thrice with equal volumes of distilled water within about forty

min. and added to 8 to 10 volumes of water. The aqueous solution was extracted thrice with ether (care was taken to avoid formation of a stable emulsion; total volume ca. 400 ml.). After evaporation of the combined ether extracts to 70 ml. in vacuo, the apparatus was filled with hydrogen and 0.5 N hydrochloric acid (700 ml.) was added to the residual solution. Then benzoyl luciferin was hydrolysed by being heated on a water bath at 100° for half an hour in hydrogen atmosphere. After cooling, the acid solution was extracted with ether to remove impurities, and then with butanol (several times, total volume ca. 200 ml.) to extract luciferin.

The modified Anderson's benzoylation method (above mentioned) was repeated again on the butanol extract. The resulting butanol solution of prified luciferin was neutralized with a saturated sodium bicarbonate solution, and evaporated in vacuo to a red residue.

In the meantime, there was prepared an apparatus for partition chromatography, which was made from a cellulose powder column (2.5×20 cm.), a hydrogen inlet tube, a funnel for addition of solvent to the upper end of the column, and three flasks at the lower end to fractionate eluates. The apparatus was first completely filled with hydrogen, and the cellulose powder column was washed with a developing solvent (ethyl acetate-ethanol-water, 5 : 2 : 3). The red residue was dissolved in a small amount of methanol (2 to 3 ml.), adsorbed on the column and then developed with the developing solvent. The developing band were as follows: 1. blue-fluorescent band, 2. faintly yellow band with bluish green fluorescence, 3. yellow band with yellow fluorescence, 4. blue-fluorescent band, 5. yellow band. The eluate of band 3 contained luciferin and was evaporated to drynes in vacuo. The residue was extracted with a small amount of methanol and evaporated again, when "purified" luciferin was obtained as an orange-colored powder.

The "purified" luciferin was dissolved in dilute methanol and centrifuged from any insoluble materials. The supernatant was acidified with hydrochloric acid and allowed to stand overnight in a desiccator in which hydrogen was substituted and somewhat depressured, upon which orange-red crystals of luciferin precipitated. Yield ca. 3 mg.

Quantitative estimation of luminescent activity of luciferin. — Determination of the luminescent activity was carried out by a modified Anderson's apparatus, which will be reported in a separate paper.

Preparation of luciferase solution for luminescent test of luciferin.—The dry residue of methanol extract of *Cypridina* (20 g.) was added to 200 ml. of water, the mixture was allowed to stand overnight and filterd. The filtrate was dialysed using cellophan for 24 hr, the dialysate was once frozen, remelted and filtered. The filtrate was diluted with water to 200 ml.

Oxidation of luciferin with ferricyanide.—To the methanol solution of luciferin was added saturated potassium ferricyanide solution, and the reaction mixture was centrifuged. The supernatant was evaporated to dryness in vacuo and the residue extracted with a small quantity of methanol to measure the ultraviolet absorption spectrum (Fig. 4).

Preparation of Oxyluciferin A and B.—One drop aqueous ammonia was added to the luciferin solution and allowed to stand overnight. The reaction mixture was evaporated to dryness and chromatographed on a cellulose powder column (solvent system; ethylacetate-ethanol-water, 5 : 2:3). Oxyluciferin A and B were eluted in separate fractions. Crystalline oxyluciferin A was obtained by evaporating fraction A, dissolving the residue in water and acidifying with hydrochloric acid; the crystals were re-crystallized from a mixture of methanol and diluted hydrochloric acid. Oxyluciferin B was not obtained crystalline. The ultraviolet absorption spectra are shown in Fig. 5. Oxyluciferin A and B are easily soluble in methanol and ethanol, but insoluble in benzene and ether.

Hydrogenation of luciferin.—After measurement of the UV absorption spectrum, the methanol solution of crystalline luciferin (ca. 50 γ in 2 ml.) was hydrogenated with ca. 0.1 mg. of Adams' platinum oxide catalyst. When the solution became almost colorless (ca. 24 hr.), the catalyst was removed by filtration and the UV absorption spectrum of the filtrate measured (Fig. 6). The amount of absorbed hydrogen could not be measured because of its minute quantity.

General procedure for hydrolysis. — *Acid hydrolysis.* — The subtsance to be hydrolysed (100—200 γ) as heated with 0.5 ml. of 4 N hydrochloric acid at 100—105° for 12 hr. in a sealed glass tube, and the hydrolysate evaporated to dryness in vacuo.

Alkali hydolysis.—The substances (100—200 γ) was heated with 0.5 ml. of 10% barium hydroxide at 100—105° for 12 hr., the hydrolysate was diluted with equal volumes of water and slightly acidified with diluted sulfuric acid. After being heated on a water bath for a few minutes, the precipitated barium sulfate was contrifuged, and the supernatant was evaporated in vacuo. The hydrolysates were analysed by means of paper chromatography.

Paper chromatography of the hydrolysates.—Toyo filter paper No. 51 was used for this purpose (ascending method). Solvent butanol-acetic acid-water (4 : 1 : 1) or 2,6-lutidine-water (3 : 2).

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SHORT COMMUNICATIONS

Tryptic Cleavage of Synthetic Poly-ε-aminocaproyl-α-amino Acids

By Mituo EBATA

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The syntheses of several linear poly-ε-aminocaproyl-α-amino acids such as poly-ε-aminocaproyl-D, L-alanine (M. W. 21,800)[1], poly-ε-aminocaproyl-L-alanine (M. W. 7,000)[2], poly-ε-aminocaproyl-L-phenylalanine (M. W. 51,200)[3], poly-ε-aminocaproyl-α-aminoisobutyric acid (M. W. 35,000)[4], poly-ε-aminocaproyl-L-leucine (M. W. 22.300)[4] and poly-ε-aminocaproylglycine (M. W. 19,300)[4] have recently been achieved by Noguchi and his coworkers, with the aid of their "N-carbothiophenylamino acid method"[5]. Among these synthetic polymers, the alanine and the α-aminoisobutyric acid derivatives, like the Bence Jones protein, possess an unusual property; they are reversibly coagulated by heat in acidic or neutral aqueous solutions[6]. In the present communication, we wish to report that crystalline trypsin of relatively high concentrations can partly hydrolyse these polymers.

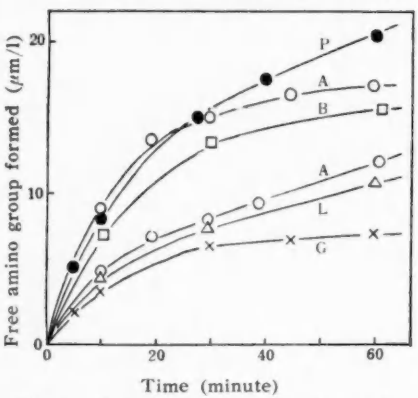

Fig. 1. Hydrolyses of poly-ε-aminocaproyl-α-amino acids by trypsin.
P, poly-ε-aminocaproyl-L-phenylalanine; A, poly-ε-aminocaproyl-D, L-alanine (above); A, poly-ε-aminocaproyl-L-alanine (bellow); B, poly-ε-aminocaproyl-α-aminoisobutyric acid; L, poly-ε-aminocaproyl-L-leucine; G, poly-ε-aminocaproylglycine.
Each reaction mixture contained 0.2 per cent of polymer, 185 μg. of twice recrystallized trypsin, and 0.013 M phosphate buffer (pH 7.1) in a final volume of 5.0 ml. Incubation was carried out at 25°.

As can be seen from Fig. 1, a considerable hydrolysis of several poly-ε-aminocaproyl-α-amino acids has occurred with 37 μg. per ml. of twice recrystallized trypsin, as evidenced in Fig. 1 by the appearance of free amino groups. The free amino groups were determined by the ninhydrin method according to Moore and Stein[7]. The viscosity of the reaction mixture decreased as the hydrolysis proceeded. No detectable hydrolysis was observed when the concentration of trypsin was reduced to 7 μg. per ml., a concentration of the enzyme suitable for its usual substrates. The polymers were resistant against the action of crystalline α-chymotrypsin and carboxypeptidase of high concentrations under the same conditions.

A search for the cleavage products of poly-ε-aminocaproyl-D, L-alanine by quantitative paper chromatography revealed that about a half mole of ε-aminocaproic acid and alanine, together with a large amount of dialysable fragments, were released from one molecule of the polymer. This suggests that the ε-aminocaproic acid and the alanine were derived from the N-terminal and the next amino acid residues of the polymer, respectively, assuming that the D-linkages were not split by the action of trypsin. The dialysable fragments yielded ε-aminocaproic acid and alanine on hydolysis with hydrochloric

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acid, and their mean degree of polymerization was estimated to be 6.

Information available up to date show that only lysyl and arginyl peptide or ester linkages are susceptible to the tryptic attack[8,9]. The results reported here, however, clearly indicate that poly-ε-aminocaproyl-α-amino acids can also be hydrolysed by the action of the proteolytic enzyme when relatively high concentrations of the enzyme are employed. It is interesting, in this connection, to note that ε-aminocaproic acid possesses a structure corresponding to α-deaminolysine. In the polymers of ε-aminocaproyl-α-amino acids, the ε-amino group, except for that of the N-terminal residue, is not in a free state. This is remarkable since the free amino groups of lysyl and arginyl residues have been believed to be required for the tryptic hydrolysis of the susceptible compounds.

Thus, the finding described above has an important bearing on the substrate specificity of trypsin and calls for a more extensive study on this point, using a number of synthetic substrates. An investigation along this line is now being carried out in our laboratory.

The author wishes to express his thanks to Professor J. Noguchi of this university, Dr. R. Sato, Osaka University and to Dr. N. Sakota, Dainippon Zoki Laboratory, for their kind encouragements throughout the course of this study.

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The Degree of Polarization of Dichroic Polarizer of Polyvinyl Alcohol-Iodine in the Region of 240–1000 mμ

By Yoshié TANIZAKI

(Received July 24, 1957)

Descriptions were given of the nature and the optical properties of several kinds of sheet polarizer[1], and of the quantitative expressions of partial dichroic polarizers[2]. But most attention was merely confined to the visible light. The present communication shows the degree of polarization, extending from 240 mμ to 1000 mμ, of a dichroic polarizer which is made by stretching the sheet of polyvinyl alcohol-iodine colored in a deep blue.

PVA (abbreviation for polyvinyl alcohol) of which the degree of polymerization was about 1500 was served to make sheet. The preparation of the sheet was described in another place[3]. The iodine solution in which the PVA sheet was soaked was prepared as the aqueous solution in the following percentages: iodine (0.1–1 g. %), ammonium iodide (2.5 times as much as iodine), sodium iodide (the same as ammonium iodide) and boric acid (5 g. %). The PVA sheet soaked in the above solution was colored in a deep blue within ten or twenty seconds. The colored sheet was stretched about five times its length in the aqueous solution of 5 % boric acid keeping about 40°C. Thus the sheet polarizer was prepared. Such a sheet looks almost neutral in color when viewed in unpolarized light, but it looks a remarkably deep blue when viewed in linerly polarized light with the electric vector parallel with the stretch direction.

Now, the degree of polarization V of the light transmitted by a single polarizer is expressed in terms of the principal transmissions k_y and k_z as follows:

$$V=\frac{k_y-k_z}{k_y+k_z} \tag{1}$$

where k_y is the transmission for the incident light completely and linerly polarized when the electric vector is at right angles to the stretch direction, and similarly k_z is the corresponding transmission when the electric vector is parallel with the stretch direction. On the other hand, for the incident unpolarized light, each of the transmissions $H_{\parallel}$ and $H_{\perp}$ of two identical partial polarizers, whose corresponding directions are respectively parallel and crossed, can be associated with k_y and k_z as follows[2];

$$H_{\parallel}=\frac{1}{2}(k_y^2+k_z^2)$$
$$H_{\perp}=k_yk_z \tag{2}$$

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Then, in this case, the degree of polarization $V(\%)$ of the light transmitted by a single partial polarizer is also given by

$$V=\left(\frac{H_{\parallel}-H_{\perp}}{H_{\parallel}+H_{\perp}}\right)^{\frac{1}{2}}\times 100(\%) \qquad (3)$$

The Beckman DU spectrophotometer was used to measure the transmissions $H_{\parallel}$ and $H_{\perp}$ according to the procedure repotred previously[3]. One of the experimental results is shown, as a typical example, by the solid lines in Fig. 1. In

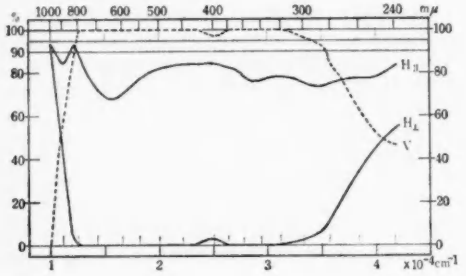

Fig. 1.

the same figure the dotted line indicates the degree of polarization, calculated from equation (3), of the light transmitted by the single sheet. The curve V shows that the degree of polarization is higher than 95% in 295–800 mμ and is almost 100% in 320–380mμ and 420–790mμ. In case of this measurement the partially polarized incident light of the spectrophotometer (e. g., about 5% at 500 mμ) was disregarded for the degree of polarization, and the correction for reflection at (four) surfaces of sheet polarizers had not been carried out. If these neglected values are taken into account, the V values all over the region will certainly become higher. It is interesting to notice that the pair of sheet polarizers, piled so as to cross their stretch directions, cuts off sharply the light in 250–800 mμ, as shown by the $H_{\perp}$ curve in Fig. 1.

This kind of sheet polarizer prepared under some conditions is not only of practical use[1] but also useful for some investigation[3], because this sheet has a high degree of polarization in the considerably broad region in wavelength and the sheet polarizer itself is comparatively stable. In our laboratory, more than two years have passed since the sheet polarizer was made and set in the filter hole of the spectrophotometer, but it has been kept in the same optical property as described above.

The author wishes to express his gratitude to Mr. T. Kobayashi, who assisted the author in the experiment, and also to Mr. T. Yoda for helpful advice regarding the preparation of the iodine solution.

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